

Communication

# First Report of Dutch Elm Disease Caused by *Ophiostoma novo-ulmi* in South Korea

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**Abstract:** During a routine survey conducted in July 2021, several dead and dying *Ulmus macrocarpa* trees were observed in an urban forest located in the Gyeonggi Province of South Korea. The trees had symptoms of wilt with yellowing and browning of leaves, and, in most cases, the trunks of dying trees were infested by bark beetles. Isolations were made from small pieces of wood taken from dying trees, and beetles were collected from the infested stems. Fungal isolates and the beetles were identified using DNA sequence-based phylogenies and morphology, respectively. The results revealed that the fungus was *Ophiostoma novo-ulmi*, the causal agent of Dutch elm disease, and the associated bark beetle was *Scolytus jacobsoni*. This study provides the first record of Dutch elm disease in South Korea and suggests that a nationwide survey for the disease should be undertaken.

**Keywords:** elm; ophiostomatoid fungi; *Ulmus macrocarpa*; *Scolytus jacobsoni*



**Citation:** Lee, D.-H.; Nam, Y.; Wingfield, M.J.; Park, H. First Report of Dutch Elm Disease Caused by *Ophiostoma novo-ulmi* in South Korea. *Forests* **2022**, *13*, 968. <https://doi.org/10.3390/f13070968>

Academic Editor: Paulo A. Zaini

Received: 25 May 2022

Accepted: 19 June 2022

Published: 21 June 2022

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## 1. Introduction

Some of the most devastating tree diseases are attributed to beetle–fungus associations, which have been recognized as major drivers of biotic damage to trees in natural and planted forests [1,2]. A relevant example in Korea is the oak wilt disease caused by *Raffaelea quercus-mongolicae* and its coevolved wood-boring ambrosia beetle vector *Platypus koryoensis* (Coleoptera; Curculionidae; Platypodidae) that led to the mass mortality of Korean oak trees, especially *Quercus mongolica*, in the country [3].

Dutch elm disease (DED) is one of the most destructive tree diseases in the Northern Hemisphere [4]. Native elm trees in Europe and North America have been devastated by two pandemics of DED caused by the pathogens *Ophiostoma ulmi* and *O. novo-ulmi*, respectively. The first of these pandemics was caused by *O. ulmi*, which spread across Europe, North America, and Southwest and Central Asia during the early 1900s. The second pandemic was caused by *O. novo-ulmi* in both Europe and North America in the 1970s [4,5]. In areas where DED currently occurs, *O. ulmi* has been replaced by *O. novo-ulmi*, which is more aggressive, leading to more destructive epidemics [6,7]. It is also relevant that *O. novo-ulmi* has two distinct subspecies known as *O. novo-ulmi* subsp. *novo-ulmi* and *O. novo-ulmi* subsp. *americana*, respectively [4,5].

The causal agents of DED are vectored by scolytine bark beetles (Curculionidae; Scolytinae) residing in the genera *Scolytus* and *Hylurgopinus* [8–10]. In addition to vector-related transmission, the DED fungi can move from infected to healthy trees by means of root grafts [11,12]. Initial symptoms of DED include wilting and discoloration of leaves on individual branches. As the disease progresses, leaves turn yellow to brown, shrivel and curl, leading to defoliation, and, eventually, entire trees die within a few weeks or a few years from the time of first infection [12,13].

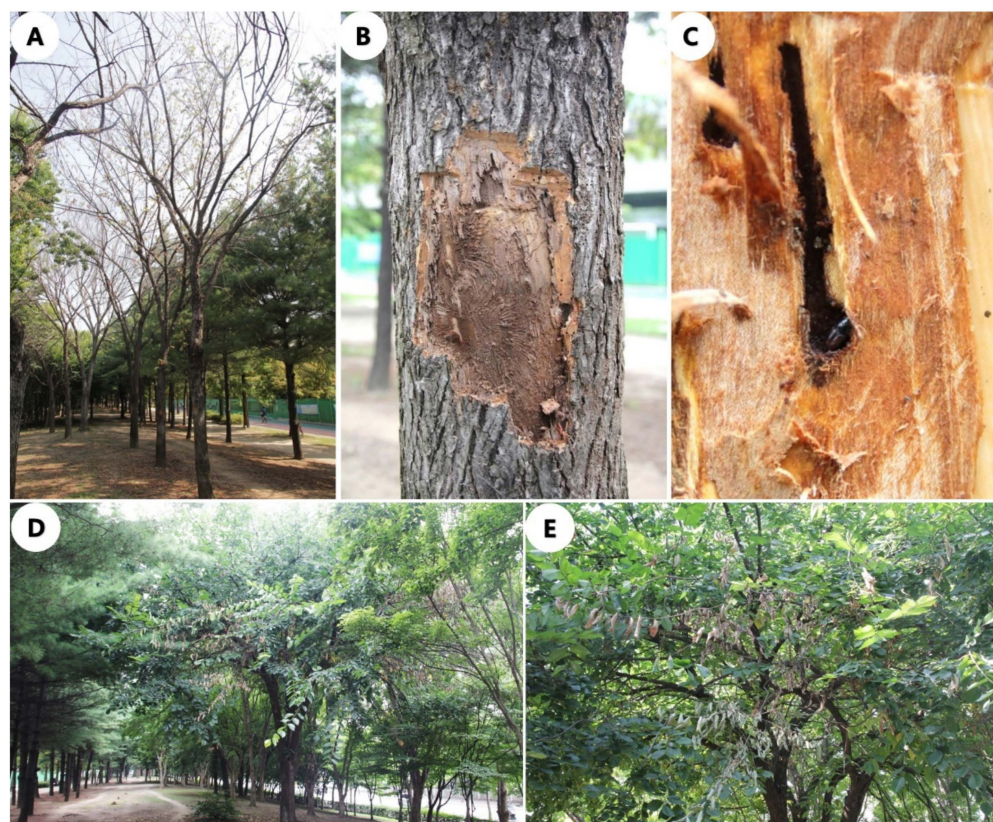
During a routine disease survey conducted in an urban forest located in Gyeonggi Province, the Republic of Korea, in July 2021, elm trees with obvious symptoms of rapid wilt were observed. Symptomatic trees were infested with bark beetles, leading to their rapid death. Although a few diseases on elm have been reported in South Korea, including leaf spot and powdery mildew [14], there have been no major health problems on elm in the country. The fact that DED had not been reported in South Korea and that elm trees were displaying wilt symptoms raised the question as to whether DED had emerged in the country. The aim of this study was, thus, to identify a possible causal agent of the disease and to consider its pathogenicity on closely related tree species.

## 2. Materials and Methods

### 2.1. Disease Symptoms, Distribution, and Identification of the Insect Vector

The distribution of dead and dying large-fruited elm (*Ulmus macrocarpa*) trees in an urban forest located in Dongducheon, a city in the Gyeonggi Province of South Korea (37°53'38.2" N 127°03'19.7" E), is currently concentrated within a narrow area approximately 700 m long. Eight dead trees and several dying trees with symptoms, including wilting, yellowing, and browning of leaves, were observed (Figure 1A,D,E). Although the dead and dying trees were relatively young, estimated at between 25 and 35 years, the disease seriously affected urban planning structures, including parks and gardens in the city.

In most cases, the trunks of dying trees were infested by bark beetles (Figure 1C), leading to beetle-breeding galleries being frequently observed (Figure 1B). Bark beetles were collected and identified based on morphology. This was achieved using a Leica dissection microscope M205C (Leica microsystems) and keys for the genus *Scolytus* [15].



**Figure 1.** Symptoms associated with DED on *Ulmus macrocarpa* in South Korea: (A) dead trees; (B) bark beetle infestation on trunk of the tree and breeding galleries developed under the bark flap; (C) insect vector *Scolytus jacobsoni* in the breeding galleries; (D) dieback with yellowing and browning of leaves; (E) close-up view of dying trees.

## 2.2. Isolation and Identification of Fungi from Bark Beetles and Wood

Tissue samples, including cambium and bark flaps, were removed from the trunks of eight dying trees for isolation. Five beetles infesting the trunks of trees were collected using an aspirator and transported to the laboratory in 1.5 mL Eppendorf tubes, where they were stored at 4 °C.

Small pieces of cambium from each of eight trees were plated on 2% malt extract agar (MEA: 20 g Difco malt extract and 20 g Difco agar) supplemented with 100 mg/L streptomycin sulfate (Sigma-Aldrich, Steinheim, Germany) after being surface sterilized. Emerging cultures were purified by transferring single hyphal tips taken from the edges of cultures to 2% MEA. The plates were incubated at 25 °C for 4 weeks in the dark and examined periodically.

Isolation from the five bark beetles was achieved by allowing them to crawl over the surface of MEA (identical to the agar used for fungal isolation). The plates were incubated at 25 °C for 2 days in the dark. The resulting cultures were purified using single hyphal tip transfers.

To check the morphology of the pathogen on medium, including synnematal production, the pure cultures were then transferred to 2% MEA in Petri dishes, to which freshly excised and subsequently 7 × 0.3 cm *U. macrocarpa* twigs sterilized by autoclaving had been placed on the agar surface. The plates were incubated following the same procedure as the isolation for cambial tissue.

## 2.3. DNA Isolation and PCR Amplifications

Two representative isolates (CDH058 and CDH059) recovered respectively from the wood sample and the beetles were cultured on 2% MEA and incubated for 2 weeks to allow sufficient mycelial growth. Mycelium was scraped from the surface of the agar with sterilized surgical scalpel blades and transferred to 1.5 mL Eppendorf tubes. Genomic DNA was then extracted using ZR Fungal/Bacterial DNA MiniPrep kit (Zymo Research, Irvine, CA, USA) following the manufacturer's instructions. The quantity and quality of DNA extracted were evaluated with a spectrophotometer (ND-1000; NanoDrop) to calibrate the concentration and purity of DNA as PCR templates.

The PCR amplifications were conducted on a T-100 thermal cycler (Bio-Rad, Hercules, CA, USA) to amplify the internal transcribed spacer (ITS) regions of rDNA using the primer set, ITS1F/ITS4 [16,17]. The total volume of each PCR reaction mixture was 15 µL, containing 1 µL of genomic DNA, 0.5 µL (10 pM) of each primer (forward and reverse), 0.5 µL of MyTaq PCR buffer (Bioline), and 0.5 µL of MyTaq DNA polymerase (Bioline). The PCR cycling profile consisted of an initial denaturation stage at 95 °C for 5 min; 35 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 1 min; and a final extension at 72 °C for 7 min. The resulting PCR products were then submitted to Cosmogenetech (Seoul, Korea) for forward and reverse sequencing reactions.

## 2.4. Pathogenicity Test

To confirm pathogenicity, a conidial suspension was prepared from the isolate obtained from wood (CDH058). The suspension was diluted to a concentration of  $1 \times 10^5$  conidia per ml and used for an inoculation trial. Three- to four-year-old saplings of *U. macrocarpa* were wounded with a 3 mm drill bit to a depth of approximately 15 mm at the root-collar region, and 10 µL of conidial suspension was placed in the wounds. In total, 19 trees were inoculated, and 8 trees served as controls (i.e., water was used instead of conidial suspension). To prevent desiccation of the inoculum and inoculation points and to reduce contamination, all inoculation points were covered with masking tape. The saplings were placed in a greenhouse located in the National Institute of Forest Science, Seoul, South Korea, for 8 weeks to allow for disease development.

### 3. Results

#### 3.1. Identification of the Insect Vector

Following the description of Park [15], the five xylophagous beetles collected from either dead or dying elm trees were confirmed to be *Scolytus jacobsoni* (Figure 2).

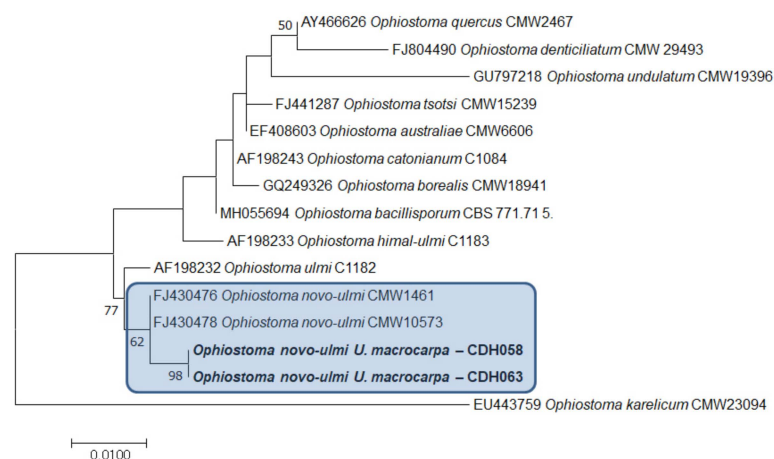


**Figure 2.** The insect vector, *Scolytus jacobsoni* (scale bar = 1 mm).

#### 3.2. Isolation and Identification of Fungi from Bark Beetles and Wood

A total of nine isolates of an *Ophiostoma* species with profuse synnematal production were recovered from the MEA where twigs of *U. macrocarpa* had been added. Five of these were from tissue samples, and the others were from beetles. All the isolates recovered in this study were deposited in the Culture Collection (CDH) of the National Institute of Forest Science, Seoul, South Korea, with accession numbers CDH058–066. Sequences of the two representative isolates, CDH058 and CDH063, that were subjected to DNA sequencing were deposited to GenBank with accession numbers ON209351–352.

A BLAST search in GenBank revealed that the sequences showed high similarity to *O. novo-ulmi* (MH055667, [18]). In addition to the BLAST result, the phylogenetic tree (Figure 3) revealed that the isolates CDH058 and CDH059 were *O. novo-ulmi*.



**Figure 3.** Phylogenetic tree based on maximum likelihood (ML) analysis. Scale bar indicates 0.01 changes.

#### 3.3. Pathogenicity Test

Symptoms, including yellowing and browning of leaves identical to those on naturally infected trees, were observed on all inoculated saplings after 8 weeks. Distinct lesions developed from the inoculated areas and the inoculated fungus were successfully reisolated from all of the dying saplings. The eight saplings inoculated with water to serve as controls remained healthy.

#### 4. Discussion

This study represents the first record of the tree pathogen *Ophiostoma novo-ulmi*, causing a vascular wilt disease on *U. macrocarpa* in South Korea. The disease was detected in a relatively small area, and the pathogen was identified based on DNA sequence comparisons. The bark beetle associated with the disease was identified as *Scolytus jacobsoni*.

*Scolytus jacobsoni* is native to Asia and is known to occur in an area stretching from southeastern Russia to northeastern China and Japan [15,19]. The beetle was first recorded in the northern Korean peninsula in 1964 [20] and later in Daegu-si, Mt. Palgong, southeastern Korean peninsula [21], suggesting that it is endemic in Korea. Whether the association of *S. jacobsoni* and *O. novo-ulmi*, as found in this study, is novel or whether beetles carrying the fungus might have recently been introduced into Korea is unknown. This important question requires further investigation.

Large-scale dieback or mortality of elm species due to DED has recently been reported in countries neighboring South Korea, including Russia [22] and Japan [23]. These are areas relatively distant from Korea, but it remains possible that these are the likely origin of the disease found in the present study. Another possible pathway of the introduction of *O. novo-ulmi* could be via introductions of infested plant material into a U.S. military camp at Donghucheon. This camp is only three kilometers away from the area where the disease described in this study occurred. However, further evidence is needed to support or reject the military pathway hypothesis and to better understand its potential for introduction within South Korea. In addition, no disease control strategies, including chemical control for DED in South Korea, have been established yet. To effectively control DED in the country, disease occurrences must be further rigorously surveyed at a nationwide scale.

**Author Contributions:** Investigation, D.-H.L., Y.N. and H.P.; resources, D.-H.L.; data curation, D.-H.L.; writing—original draft preparation, D.-H.L. and Y.N.; writing—review and editing, M.J.W.; funding acquisition, D.-H.L. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was supported by a project, Investigation and characterization on occurrence of emerging forest insect pests and fungal diseases (Project No. FE0702-2021-01), from the National Institute of Forest Science, Republic of Korea.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Acknowledgments:** We are grateful to Sang-Wook Park for assistance in identifying the bark beetle *Scolytus jacobsoni*.

**Conflicts of Interest:** The authors declare no conflict of interest. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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