

Macruropyxis fraxini on *Fraxinus rhynchophylla*: Confirmation in the Korean Peninsula after 82 Years and the First Record in South Korea

Bok-Nam Jung^a , Young-Joon Choi^b , Hyeon-Dong Shin^c  and Ji-Hyun Park^a 

^aDivision of Forest Insect Pests and Diseases, National Institute of Forest Science, Seoul, Korea; ^bDepartment of Biology, Kunsan National University, Gunsan, Korea; ^cDivision of Environmental Science and Ecological Engineering, College of Life Sciences and Biotechnology, Korea University, Seoul, Korea

ABSTRACT

Macruropyxis fraxini has been recorded on several species of *Fraxinus* in China, Japan, Russia (Far East), and North Korea since its first recorded observation as a rust fungus on *F. rhynchophylla* in Jilin, China, in 1899. In the Korean Peninsula, the rust fungus was first recorded on *F. rhynchophylla* in 1935, based on four specimens collected at Mt. Kungangsan, Gangwondo Province, in the North Korean territory. We confirmed this rust in the Korean Peninsula after 82 years. The rust fungus was identified based on morphological characteristics and a molecular phylogenetic analysis. This is the first record of *M. fraxini* in South Korea.

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Fraxinus rhynchophylla Hance, commonly known as the East Asian ash, grows in moist, fertile soils on hillsides and in river valleys in China, Korea, and Japan (<https://species.nibr.go.kr>). In Korea, *F. rhynchophylla* is a species that naturally regenerates and dominates after thinning, thereby promoting the restoration of native hardwood forests. The species is also an important wildlife habitat, stabilizes stream banks, and provides organic matters to the forest. Its wood is commonly used for the manufacture of furniture, sports equipment, and tool handles, owing to its hard, dense, but elastic properties. In addition, its bark contains useful chemical compounds, and has thus long been utilized for medicinal purposes in Asia [1].

Three species of rust fungi have been recorded on *Fraxinus* spp. globally (<http://nt.ars-grin.gov/fungal-databases>). *Puccinia fraxinata* (Link) Arthur has been recorded on more than five species of *Fraxinus*, exclusively in the United States, since it was first recorded in 1825 as *Caeoma fraxinatum* Link. *Aecidium fraxini-bungeanae* Dietel was recorded on *F. bungeana* var. *pubinervis* (Blume) Wenz. in Japan in 1903, but no further studies have been conducted on its identity [2,3]. *Macruropyxis fraxini* (Kom.) Azbukina has been recorded on six species of *Fraxinus*, all of which are native to East Asia, including the Far East of Russia, China, Korea, and Japan [4].

In Korea, Hiratsuka first recorded *M. fraxini* on the basis of four rust materials on *F. rhynchophylla* collected in 1934 at Mt. Kungangsan (currently within the North Korean territory, approximately 38°39'N 128°11'E) [5]. Since that time, there have been no additional records of this rust fungus on *F. rhynchophylla* in the Korean Peninsula [6]. During our extensive forays for phytopathogenic fungi, for the first time in South Korea, we found a rust fungus on the leaves of *F. rhynchophylla* at Hongcheon in 2016, and again at Hongcheon and Chuncheon in 2017 and 2018.

Voucher specimens of the leaves of *F. rhynchophylla* have been deposited in the Korea University Herbarium: KUS-F29396 (September 1 2016, Hongcheon), KUS-F29991 (September 2 2017, Chuncheon), KUS-F30071 (September 26 2017, Hongcheon), and KUS-F30810 (September 10 2018, Chuncheon).

To perform morphological observations, the fungal structures were detached from the pustules using watchmaker's forceps and water-mounted on glass slides. The morphology of the telia, teliospores, and pedicels was examined using a bright field and differential interference contrast (DIC) light microscope (Olympus BX51; Olympus, Tokyo, Japan) and a Zeiss AX10 microscope equipped with an AxioCam MRc5 camera (Carl Zeiss, Oberkochen, Germany). The microstructure of telia and

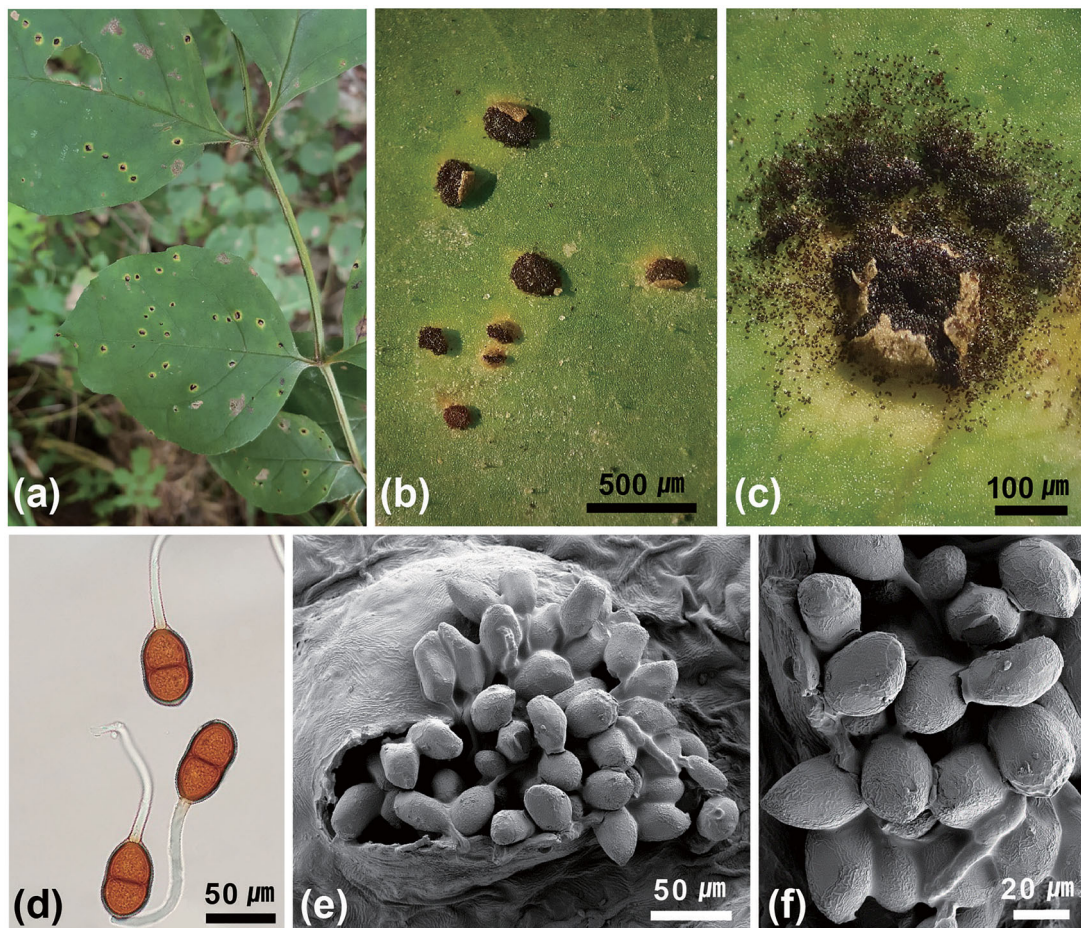


Figure 1. *Macruropyxis fraxini* on *Fraxinus rhynchophylla*. (a) Symptoms on the adaxial leaf surface. (b) Telia developing on the abaxial leaf surface, surrounded by a yellow halo. (c) Magnified view of an erumpent telium. (d) Median view of teliospores under a light microscope. (e and f) A telium showing a group of teliospores (e) and close-up of teliospores (f) observed under a scanning electron microscope.

teliospore was observed using a field-emission scanning electron microscope (SUPRA 55VP; Carl Zeiss).

Rust pustules on leaf surfaces were circular, subcircular to irregular, brown to dark-brown, crust-like, and granular (Figure 1(a)). Telia were predominantly formed on the adaxial surface of leaves but rarely also found on the abaxial leaf surface and young stems, exposed, chestnut-brown to dark-brown, and 100–300 μm in diameter, occasionally reaching 1000 μm ($n=30$) (Figure 1(b,c)). Teliospores were ellipsoid, 36–57 \times 25–38 μm , 2-celled, inner wall brown, 4–6 μm thick, outer wall hyaline, smooth, germ pores 2–4 per cell; pedicels were hyaline, composed of cell walls with loss of cytoplasm, 4.5–6.5 μm wide, and up to 120–280 μm long ($n=30$) (Figure 1(d–f)). These morphological features were consistent with the description of *M. fraxini* on *Fraxinus* spp. reported by Azbukina in 1972 [4], except for a slight difference in the size of teliospores, viz., 35–55 \times 23–35 μm . Spermogonial, aecial, and uredinial stages were not observed in both studies.

Genomic DNA was extracted from the rust pustules harvested from infected leaves and amplified with the rust-specific primer sets Rust2inv/ITS4rust, Rust2inv/LR6, and LRust1R/LR6 [7]. An approximate 1400-bp region of the 5.8S subunit, the internal transcribed spacer 2 (ITS2), and large-subunit (28S) were sequenced. The assembled sequences were compared with entries in the NCBI database using the BLASTn program. For phylogenetic analysis, the combined sequences of the 5.8S, ITS-2, and 28S regions of the rDNA were initially aligned with rust species retrieved from GenBank and manually edited. *Cronartium ribicola* was used as an outgroup taxon (GenBank Accession No. HQ31709). A phylogenetic tree was constructed using MEGA7 [8] based on neighbor-joining (NJ) analysis. Gaps in the sequence alignment were treated as deletions. The strength of the internal branches of the resulting tree was determined with a bootstrap analysis of 1000 replications.

The ITS and LSU sequences obtained from our two specimens (KUS-F29991 and KUS-F30071) have been deposited in GenBank (ITS: MT126442 and MT126443, LSU: MT126444 and MT126445). In a

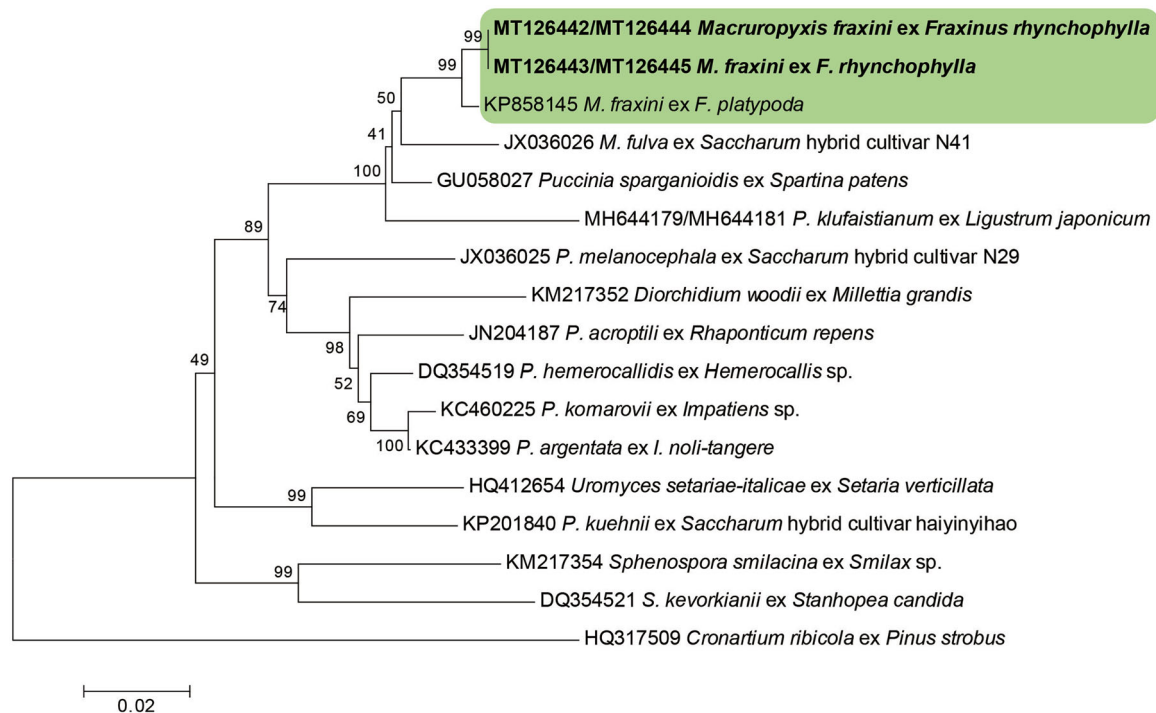


Figure 2. Phylogenetic relationship between *Macruropyxis fraxini* and related rust fungi retrieved from GenBank, inferred by the neighbor-joining (NJ) method using the 5.8S, ITS2, and partial LSU rDNA sequences. Bootstrap values based on 1000 replications are indicated at the branches. The scale bar represents 0.02 nucleotide substitutions per site. The Korean isolates described in this study are shown in bold.

BLASTn analysis, they showed high similarities (ITS: 99.08% and LSU: 99.31%) with *M. fraxini*. Our phylogenetic analysis indicated that the combined sequences of the ITS and LSU regions clustered in the same clade with the reference sequence of *M. fraxini* parasitic to *F. platypoda* (Figure 2).

The genus *Macruropyxis* was established by Azbukina to accommodate *Uropyxis fraxini*, which was originally designated as *Puccinia fraxini* by Komarov in 1899. *M. fraxini* (Kom.) Azbukina had been the only species in this genus until the inclusion in 2017 of *M. fulva*, a rust fungus infecting sugarcane in southern Africa [9]. Nevertheless, the identity of the genus *Macruropyxis* remains contentious, as to whether it should be accorded a distinct genus status or be included in the genus *Puccinia* [7]. Therefore, more information on diverse isolates of *M. fraxini* would contribute to solving this ambiguity.

M. fraxini has been collected from five species of *Fraxinus*, including *F. rhynchophylla* (<http://www.indexfungorum.org>) exclusively in East Asia. Hiratsuka first recorded *Uropyxis fraxini* in the Korean Peninsula on the basis of rust-infected *F. rhynchophylla* collected at Mt. Kumgangsan. Since that time, there have been no additional collections or records of ash rust in North Korea [6]. The description reported herein is an official record of *M. fraxini* confirmed in the Korean Peninsula after 82 years and is the first record of this species in South Korea. Information on the ITS and LSU

sequences, as well as the morphological characteristics of the South Korean isolates, will contribute to our understanding of the generic concept of *Macruropyxis* and the species identity of *M. fraxini*.

Disclosure statement

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

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ORCID

Bok-Nam Jung  <http://orcid.org/0000-0002-5981-0492>

Young-Joon Choi  <http://orcid.org/0000-0002-0909-4723>

Hyeon-Dong Shin  <http://orcid.org/0000-0002-7778-2747>

Ji-Hyun Park  <http://orcid.org/0000-0002-6778-4749>

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