

RESEARCH NOTE

New Finding of *Golovinomyces salviae* Powdery Mildew on *Glechoma longituba* (Lamiaceae), Besides Its Original Host *Salvia* spp.

In-Young Choi^{1,†}, Lamiya Abasova^{1,†}, Joon-Ho Choi¹, Young-Joon Choi², Hyeon-Dong Shin^{1,3*}

¹Department of Agricultural Biology, Jeonbuk National University, Jeonju 54896, Korea

²Department of Biological Science, Kunsan National University, Gunsan 54150, Korea

³Division of Environmental Science and Ecological Engineering, Korea University, Seoul 02841, Korea

*Corresponding author: hdshin@korea.ac.kr

†These authors contributed equally to this study

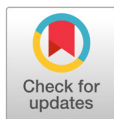
ABSTRACT

The *Golovinomyces biocellatus* complex is known to consist of powdery mildew from the *Golovinomyces* genus, associated with host plants from the Lamiaceae family. Recent molecular phylogenetic analyses have resolved the taxonomic composition of this complex, and *Golovinomyces biocellatus* sensu stricto is considered to be a pathogen of *Glechoma* species, globally. However, this paper presents a new finding of *Golovinomyces salviae* on *Glechoma longituba*, besides its original host species of *Salvia*. This information was inferred by molecular phylogenetic analyses from the multi-locus nucleotide sequence dataset of intergeneric spacer (IGS), internal transcribed spacer (ITS), large subunit (LSU) of rDNA, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene. Further, the asexual morphology of this fungus is described and illustrated.

Keywords: Erysiphaceae, *Golovinomyces biocellatus*, GAPDH (glyceraldehyde-3-phosphate dehydrogenase), ITS (internal transcribed spacer), LSU (large subunit), Multigene analysis

Glechoma longituba (Nakai) Kuprian. (syn. *Glechoma hederacea* var. *longituba* Nakai) of the Lamiaceae family, known as long tube ground ivy, is native to Vietnam, China, Korea, and the Russian Far East (<https://powo.science.kew.org/>). This plant has been used for medicinal purposes in Asia since long [1,2]. In Korea, these plants are dried and marketed as herbal tea [3].

Powdery mildew infections of *Glechoma* L. species are known to be associated with *Golovinomyces biocellatus* (Ehrenb.) V. P. Heluta [4]. In Korea, this pathogen is associated with *Agastache rugosa* Kuntze, *Meehania urticifolia* Makino, *Mentha suaveolens* Ehrh., *Monarda citriodora* Cerv. ex Lag., *M. didyma* L., and *Rosmarinus officinalis* L. from the Lamiaceae family [5-10]. Powdery mildew from the *Golovinomyces* genus associated with host plants of the Lamiaceae family are known to be a part of the *G. biocellatus* complex [11]. Recent molecular phylogenetic analyses have resolved the taxonomic



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composition of this complex by categorizing it into several new combinations, including *Golovinomyces monardae* (G.S. Nagy) M. Scholler, U. Braun & Anke Schmidt, *G. neosalviae* M. Scholler, U. Braun & Anke Schmidt, and *G. salviae* (Jacz.) M. Scholler, U. Braun & Anke Schmidt [12]. Currently, *Golovinomyces bicellatus* sensu stricto is considered to be a pathogen of *Glechoma* and *Lycopus* L. species.

During routine plant disease surveys in 2022, *G. longituba* plants were found to be infected with powdery mildew in Jeongeup, Jeonju, Buan, Jinan, and Wanju, Korea. Subsequently, six samples were deposited in the Mycological Herbarium of Korea University, namely KUS-F32257 (June 15, 2021, Hongcheon), F32837 (May 20, 2022, Jeongeup), F32916 (June 7, 2022, Jeonju), F32934 (June 10, 2022, Wanju), F32977 (June 20, 2022, Buan), and F33540 (November 13, 2022, Jinan).

For morphological examination, a small piece of the fungal colony was scraped off the infected leaves and mounted on a drop of distilled water on a glass slide. Subsequently, they were examined under an optical microscope using bright-field and differential interference contrast (DIC). At least 30 measurements of each structure were taken using an Olympus BX51 microscope (Olympus, Tokyo, Japan) at 40 \times and 100 \times magnifications, and a Zeiss AX10 microscope equipped with an AxioCam MRc5 (Carl Zeiss, Göttingen, Germany) was used for capturing the photographs.

Powdery mildew colonies first appeared as circular to irregular white patches but soon progressed to abundant hyphal growth on both sides of the leaves and young stems (Fig. 1A and B). Severe infection caused the withering and premature senescence of leaves in plants, reducing their vigor. In powdery mildew, the hyphae were septate, branched, and 4-9 μm wide, and the hyphal appressoria were nipple-shaped. Conidiophores measured 120-200 \times 10-12 μm and produced 1-4 immature conidia in chains with a sinuate outline, followed by 2-3 cells (Fig. 1C-E). The foot-cells of conidiophores were straight, cylindrical, and 35-55 μm long. Conidia were hyaline, were ellipsoid to barrel-shaped, measured 22-30 \times 19-23 μm (length/width ratio 1.1-1.6), lacked distinct fibrosin bodies, and produced germ tubes at the subterminal position (Fig. 1F). Chasmothecia were not observed. Short conidiophores and a low length/width ratio of conidia are typical characteristics indicating the asexual state of *Golovinomyces salviae* [12].



Fig. 1. Powdery mildew caused by *Golovinomyces salviae* on *Glechoma longituba*. A, B: Symptoms on the leaves. C-E: Conidiophores. F: Conidia.

For molecular phylogenetic analyses, genomic DNA was extracted from the mycelia of three samples (i.e., KUS-F32837, F32916, and F32934) using Maglisto™ 5M kits (Bioneer, Daejeon, Korea), following the manufacturer's instructions. The nucleotide sequences of the internal transcribed spacer (ITS) and intergeneric spacer (IGS) regions as well as the large subunit (LSU) of the rDNA, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene were amplified and sequenced using the primer pairs ITS1/PM6, IGS-12a/NS1R, PM3/TW14, and PMGAPDH1/PMGAPDH3R, respectively [13,14]. Newly obtained sequences were assembled and deposited in the NCBI database under the accession numbers OP872648, OP872652, and OP877276 for ITS; OP872651, OP872653, and OP872655 for LSU; OQ281744-OQ281746 for IGS; and OQ281747-OQ281749 for GAPDH. These sequences were then combined in a multigene dataset of GAPDH+IGS+ITS+LSU with 33 *Golovinomyces* sequences retrieved from GenBank, according to method described by Bradshaw et al. [14]. Following this, they were aligned using the MUSCLE command implemented in MEGA 11 [15]. *Neocrysiptis galeopsidis* (DC.) U. Braun was selected as the outgroup. A phylogenetic tree was generated based on the maximum parsimony (MP) method in PAUP* 4.0a, with the heuristic search option using the 'tree-bisection-reconstruction' (TBR) algorithm. All sites were treated as unordered and unweighted, and gaps were treated as missing data [16]. The robustness of the internal branches was evaluated using bootstrap (BS) analysis with 1,000 replications. The tree scores calculate were tree length (TL), consistency index (CI), retention index (RI), and rescaled consistency index (RC).

The resulting sequences of ITS were 100% identical to those of *G. salviae* (AB769437 and LC100001 etc.) and *G. biocellatus* (AB307669), whereas sequences of LSU showed 99.87% similarity with *G. salviae* (LC076803 and LC100001). The GAPDH and IGS results showed 99.6% and 99.4% similarity to *G. salviae* (ON075654 and ON075655), respectively. In total, 37 sequences and 1,876 characters were used in the MP analysis. Among the 1,876 characters, 1,491 (79.4%) were constant, 281 (14.9%) were parsimony-informative, and 104 (5.54%) were variable and parsimony-uninformative. In the consensus tree (Fig. 2), the sequences obtained from the three Korean isolates were clustered in a distinct clade with the sequences of *G. salviae*, and the branch was supported with a 100% bootstrap value.

Glechoma species belong to the Menthaeae tribe of the Lamiaceae family. According to a study on *Golovinomyces biocellatus* complex conducted by Schoeller et al. [12], most host plant species from this tribe, such as *Mentha* L., *Monarda* L., *Origanum* L., and *Thymus* L. spp., are infected by *Golovinomyces monardae*. In contrast, *Glechoma hederacea* L. has been reported to be a target host plant for *G. biocellatus* sensu stricto. However, *G. salviae* was reported as a new combination that was excluded from the biocellatus complex, owing to its pathogenicity to *Salvia* spp. (tribe Menthaeae) in Europe. Recently, *Agastache scrophulariifolia* (Wild.) Kuntza, from the Menthaeae tribe, has been recorded as the host plant of this fungus in the USA [14]. Our research suggests that the host-plant range of *G. salviae* is increasing, and the target host plants of powdery mildew pathogen (from the Menthaeae tribe) are not confined to one species, but includes several *Golovinomyces* species. To the best of our knowledge, this is the first report of powdery mildew caused by *G. salviae* on *G. longituba*, and the first report of powdery mildew in Korea.

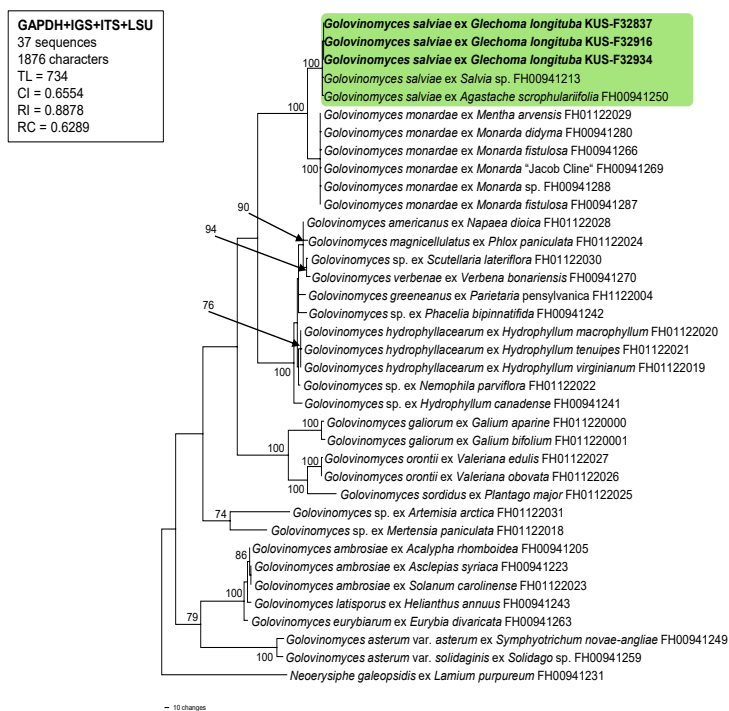


Fig. 2. A phylogenetic tree of *Golovinomyces* species is generated from a combined multigene dataset of GAPDH+IGS+ITS+LSU sequences based on the maximum parsimony method. *Neoerysiphe galeopsidis* was used as an outgroup. The newly obtained sequences are shown in bold. Bootstrap values (>70%) are indicated on related branches. Tree scores such as tree length (TL), consistency index (CI), retention index (RI), and rescaled consistency index (RC) are given in the box. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IGS, intergeneric spacer; ITS, internal transcribed spacer; LSU, large subunit.

CONFLICT OF INTEREST

No potential conflict of interest is reported by the authors.

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