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Chalaropsis pruni sp. nov. (Ceratocystidaceae), a new species from *Prunus serrulata* var. *pubescens* in South Korea

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Abstract

A routine survey in a natural forest located in the Gangwon Province of Korea was undertaken in 2022 to establish an inventory of potentially pathogenic fungi that might affect tree health in the country. An ophiostomatoid fungus was consistently isolated from naturally occurring wounds on *Prunus serrulata* var. *pubescens*. Isolates were subjected to morphological and DNA sequence comparisons. The results showed that the fungus resided in a distinct taxonomic lineage, and the novel species is described here as *Chalaropsis pruni sp. nov.*

Key words: 1 new species, Microascales, ophiostomatoid, *Prunus*, wound

Introduction

The ascomycete family Ceratocystidaceae includes a diverse and widely distributed group of fungi (Seifert *et al*. 2013, de Beer *et al*. 2014, Wijayawardene *et al.* 2022). They have diverse lifestyles including saprotrophs and plant pathogens that are most commonly transmitted by insects including nitidulid beetles (Coleoptera: Nitidulidae) and flies (Diptera) (Moller & DeVay 1968, Heath *et al*. 2009). Many also include important plant pathogens especially of root crops and trees (Kile 1993, Roux & Wingfield 2013, de Beer *et al*. 2014). They typically infect their hosts through wounds, and can cause fruit and tuber rot as well as canker and wilt diseases of woody plants (Kile 1993, Roux & Wingfield 2009).

Genera in the Ceratocystidaceae are typically characterized by their mostly black, globose ascomatal bases with elongated necks terminating in an ostiole, through which sticky ascospores exude (Upadhyay 1981, Seifert *et al*. 1993, de Beer *et al*. 2014). In most cases, this group can be futher characterized by asexual forms that produce chains of rectangular or barrel-shaped endoconidia produced from phialidic conidiophores and, in some cases, dark-walled aleurioconidia (de Hoog & Scheffer 1984, de Beer *et al*. 2014).

A routine survey in natural forests located in Gangneung (Gangwon province, the Republic of Korea) was conducted in 2022. The aim was primarily to establish an inventory of potentially pathogenic fungi that might affect the health of *Prunus* spp. in the future. As part of this study, fungi were isolated from wounds on trees resulting from severe storm damage during the summer of 2022. The isolated fungi included a *Chalaropsis* sp. that consistently emerged from wounds on *Prunus serrulata* var. *pubescens* (mountain oriental cherry). The aim of this study was to identify the fungus and to compare it with known *Chalaropsis* spp.

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Materials and methods

Isolation

The *Chalaropsis* sp. was collected from fresh wounds on *P. serrulata* var. *pubescens* growing in natural forests located in Gangneung, a city in the Gangwon Province of South Korea (37°43'41.7"N 128°47'56.1"E, 37°43'37.4"N 128°47'53.6"E) during July and August, 2022. Pieces of bark bearing fungal mats were placed in individual paper bags and transported to the laboratory for further study.

Isolations were made on 2% malt extract agar (MEA; 20 g malt extract, Difco; 20 g agar, Difco) supplemented with 100 mg L^{-1} streptomycin sulphate (Sigma-Aldrich) by transferring spore drops at the apices of the ascomata to the medium. Cultures were incubated at 25 °C for two weeks in the dark. All the isolates recovered in this study were deposited in the Culture Collection (CDH) of the Chungnam National University, Daejeon, Republic of Korea (Accession Nos. CDH061 and CDH062). The holotype specimen, CDH061, was deposited in the herbarium collection (KH) of Korea National Arboretum, Pocheon, South Korea (Accession No. KA24-0002), and the ex-holotype culture was deposited in the Korean Agricultural Culture Collection (KACC) of the National Academy of Agricultural Science, Jeonju, South Korea (Accession No. KACC 410708).

Microscopy

Fungal structures were mounted on microscope slides in water that was later replaced with 85% lactic acid for further observation. The structures were examined using a Zeiss AX10 Imager A2 (Carl Zeiss Microscopy GmbH, Göttingen, Germany) equipped with an Axiocam 506 digital camera. Up to 30 measurements were taken for taxonomically relevant structures when this was possible.

Genomic DNA extraction, PCR amplification, and sequencing

To extract genomic DNA, cultures were incubated for two weeks to allow for 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 the DNA extracted was evaluated with a spectrophotometer (ND-1000; NanoDrop) to calibrate the concentration and purity of DNA as PCR templates.

The PCR amplification reactions were conducted on a T-100 thermal cycler (Bio-Rad, CA, USA). 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 gene regions that were subjected to sequencing and phylogenetic analyses included ribosomal large subunit (LSU), the 60S ribosomal protein RPL10 (60S), the internal transcribed spacer region (ITS), and the minichromosome maintenance complex component 7 (MCM7). The LSU, 60S, ITS and MCM7 regions were amplified using the primers LR0R / LR5 (Vilgalys & Hester 1990), ITS1F / ITS4 (Gardes & Bruns 1993, White *et al*. 1990), 60S-506F / 60S-908R (Stielow *et al*. 2015), and MCM7-for / MCM7-rev (de Beer *et al*. 2014), respectively. The resulting PCR products were submitted to Macrogen (Seoul, Korea) for forward and reverse sequencing reactions.

Multi-gene phylogenetic analyses

The sequences of *Chalaropsis* spp. closely related to the one from *P. serrulata* var. *pubescens* were retrieved from GenBank. Phylogenetic trees based on a concatenated data set of the LSU, 60S, ITS, and MCM7 were computed. Sequences for each of the four gene regions were aligned using the online interface of MAFFT ver. 7 (http://mafft.cbrc.jp/alignment/server) (Katoh *et al*. 2002), with the iterative refinement method (FFT-NS-i settings) selected. Sequence alignments were manually edited in MEGA7 (Kumar *et al*. 2016). Two different phylogenetic analyses were employed, including maximum parsimony (MP) analyses using MEGA7 and maximum likelihood (ML) tests using RAxML HPC BlackBox ver. 8.1.11 (Stamatakis 2006, Stamatakis *et al*. 2008), using the default option with the GTR substitution model implemented in the CIPRES cluster server (https://www.phylo.org/) at the San Diego Supercomputing Center. For both MP and ML analyses, *Thielaviopsis* spp. were used as the outgroup taxon. The introduction of the new species follow the guidelines of Maharachchimbukura *et al.* (2021).

Results

Multi-gene phylogenetic analyses and sequence comparisons

Four gene regions, (LSU, 60S, ITS, and MCM7), were successfully sequenced, and these were deposited in GenBank with accession nos. PP392802-803 for LSU, PP395622-623 for 60S, PP392800-801 for ITS, and PP395624-625 for MCM7. The sequences obtained for the LSU, 60S, ITS, and MCM7 gene regions were aligned with those of closely related *Chalaropsis* spp., based on the BLAST search results from the NCBI nucleotide database. Phylogenetic analyses using the concatenated LSU, 60S, ITS, and MCM7 gene sequences resulted in a tree (Figure 1). Although the overall topologies generated from both ML and MP analyses were slightly different from each other, they consistently showed that the isolates from *P. serrulata* var. *pubescens* were of a previously undescribed species. This species was most closely related to *Chalaropsis thielavioides* (Nag Raj & Kendrick 1975), but it was distinct from that species and all previously described *Chalaropsis* spp.

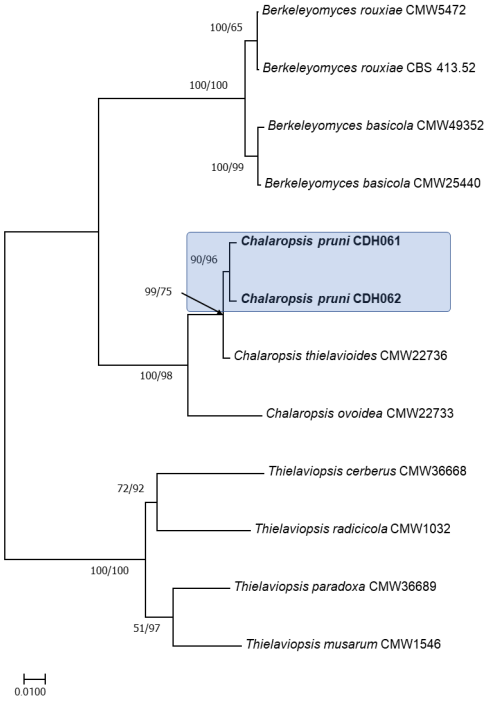

FIGURE 1. Phylogenetic trees based on maximum likelihood (ML) analysis of datasets of a combined dataset of ITS, LSU, 60S and MCM7 gene sequences for *Chalaropsis* species and its close related taxa residing in Ceratocystidaceae. Isolates in bold and highlighted are the new species of *C. pruni* described in this study. Bootstrap values >50% for MP and ML are presented above branches as MP/ML, bootstrap values absent are not shown. Scale bar indicates 0.01 changes.

Sequence comparisons revealed that the undescribed species differed from *C. thielavioides* at 1 of 820 characters (about 0.1%) in the LSU (Accession no. KM495402), at 4 of 385 characters (about 1.0%) in the 60S (Accession no. KM495579), at 2 of 396 characters (about 0.5%) in the ITS (Accession no. FJ411342), and at 6 of 616 characters (about 1.0%) in the MCM7 sequences (Accession no. KM495489).

Taxonomy

Morphological comparisons and phylogenetic inference based on four gene regions provided sufficient evidence that the *C. pruni* isolated from freshly made wounds on *P. serrulata* var. *pubescens* represents an undescribed species residing in the Ceratocystidaceae. This species is described as follows:

Chalaropsis pruni D.Hyeon Lee & S.E. Cho, *sp. nov.* (Figure 2)
MycoBank No. MB 854086

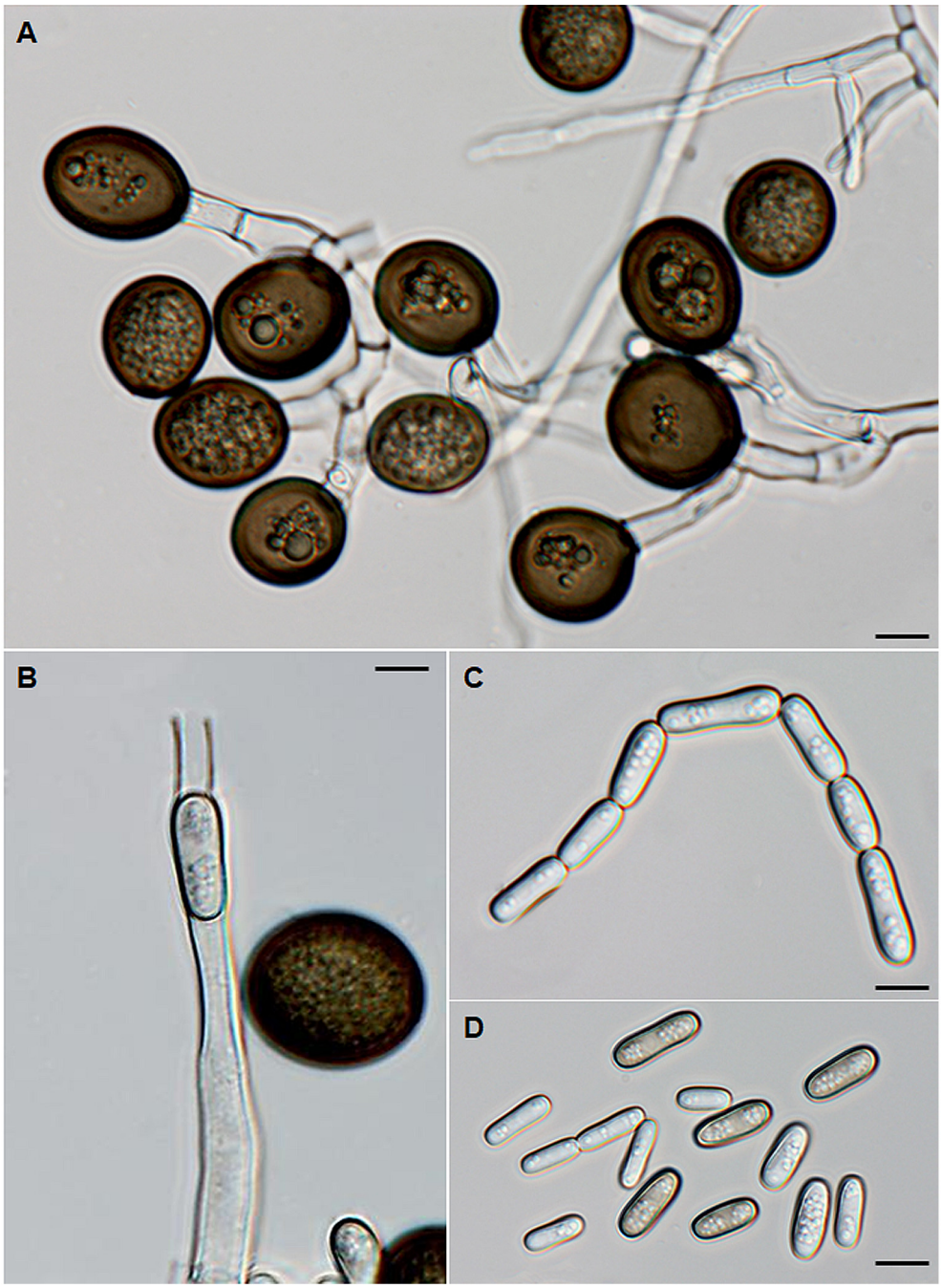

FIGURE 2. Microscopic features of *Chalaropsis pruni sp. nov.* (ex-holotype: KACC 410708). A. Chlamydospores, B. Terminal phialide giving rise to an endoconidium, C. Conidia produced in chain, D. Conidia. Scale bar = 10 µm.

Etymology: The epithet refers to the host genus *Prunus*, from which the fungus was collected.

Description: *Conidiophores* phialidic, cylindrical, hyaline, tapering toward apex, 3.8–4.2 µm at the tip and 8.9–10.8 µm at the base. *Chlamydospores* unicellular, globose, dark-brown, 18–21 × 19–23 µm. *Conidia* unicellular, cylindrical, hyaline, produced singly or in chains, 8.1–22.3 µm long × 3.9–5.3 µm wide.

Culture characteristics: Colonies on MEA initially white, becoming dark green. Mycelium immersed and superficial. Hyphae smooth, septate. Optimal temperature for growth 25 °C reaching 75 mm in 14 days.

Typus: Mt. 293-1, Eoheul-ri, Seongsan-myeon, Gangneung-si, the Gangwon Province of South Korea (37°43'41.7"N 128°47'56.1"E), isolated from fresh wounds (less than one month-old) on *Prunus serrulata* var. *pubescens*. The holotype, KA24-0002, dried culture of CDH061, was deposited in the herbarium collection (KH) of Korea National Arboretum Korea. The ex-holotype culture (CDH061 = KACC 410708) was deposited in the culture collection (CDH) of the Chungnam National University and Korean Agricultural Culture Collection (KACC) of the National Academy of Agricultural Science for Type Cultures.

Additional specimen examined: Mt. 293-1, Eoheul-ri, Seongsan-myeon, Gangneung-si, the Gangwon Province of South Korea (37°43'41.7"N 128°47'56.1"E), isolated from fresh wounds on *P. serrulata* var. *pubescens*, August 2022, D.H. Lee, culture CDH062.

Habitat: Fresh wounds (less than one month-old) on *P. serrulata* var. *pubescens* trees.

Known distribution: The Republic of Korea (Gangwon Province)

Notes: *Chalaropsis pruni* is phylogenetically closely related to *C. thielavioides.* It can, however, be distinguished from that species by its slightly larger chlamydospores and smaller conidia, which are 9–19 µm long × 7.5–18 µm wide and 6.5–32 µm long × 2.5–6.5 µm wide, respectively (Nag Raj & Kendrick 1975).

Discussion

A routine tree health survey in a natural forest of Korea resulted in the discovery of a new *Chalaropsis* sp. commonly occurring on fresh wounds on *P. serrulata* var. *pubescens.* Phylogenetic inference based on sequence data for parts of LSU, 60S, ITS, and MCM7 gene regions showed that the fungus is a novel species of *Chalaropsis* for which we have provided the name *Chalaropsis pruni*. *Chalaropsis pruni* was most closely related to *C. thielavioides*, and this species can easily be distinguished from that and other *Chalaropsis* spp. based on its morphological characteristics and DNA sequences.

The presence of *C. pruni* on wounds of trees is typical for various other genera in the Ceratocystidaceae as defined by de Beer *et al*. (2014). We assume that, as with species in this family (Moller & DeVay 1968, Heath *et al*. 2009), *C. pruni* was transferred to the wounds by insects such as flies or nitidulid beetles. Future studies will focus on identifying its possible vectors in Korea.

The asexual genus *Chalaropsis*, first established by Peyronel (1916), was recently revised to incorporate three species that inhabit woody substrates by de Beer *et al*. (2014). This was based on *C. thielavioides*, which had previously been known as *Chalara thielavioides* and *Thielaviopsis thielavioides*. While there are some instances of fungi in this group and mostly *C. thielaviodes* causing diseases such as wilt on rubber trees (Li *et al*. 2021) and postharvest rot on carrot (Xu *et al*. 2020), very little is known regarding the economic importance or ecological significance of *Charlaropsis* spp. It is likely that *C. pruni* is an early colonsist of fresh stem wounds on *P. serrulata* var. *pubescens* trees. While there was no evidence of trees dying due to its presence, it could contribute to tree disease. In this regard, pathogenicity tests should be undertaken with *C. pruni* to determine its relative importance.

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