Dissertation Title Taxonomy and Phylogeny of Microfungi Associated with *Amomum Villosum* in Southern China
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ABSTRACT

Wurfbainia villosa (=*Amomum villosum*), commonly known as "Sharen or Chunsharen" in Chinese, is a perennial, evergreen herb, known as one of the "four great southern medicines" in China. *Wurfbainia villosa* is extensively cultivated in Yunnan and Guangdong provinces, China, owing to its crucial medicinal properties and its essential role as a culinary spice. However, the taxonomic and phylogenetic research on microfungi associated with *W. villosa* remains highly limited. To date, only 18 fungal species have been documented on *W. villosa*, which significantly constrains our understanding of its fungal diversity. Thus, our objective is to conduct extensive, long-term surveys to comprehensively investigate the microfungi on *W. villosa* in southern China and to establish a global checklist of fungi associated with this plant. Based on the morphological and phylogenetic evidence (ITS, LSU, SSU, *act*, *chs-1*, *gapbh*, *rpb1*, *rpb*2, *tub*2, *tef1-α*), a total of 77 species were identified, which are distributed across 32 families in Sordariomycetes, Dothideomycetes, Eurotiomycetes, Agaricomycetes, and Mucoromycetes. Fungal taxa were discovered from 41 genera, including two new genera, 47 new species, and 30 new host and geographical records, and a species pending confirmation. Two new genera are *Bulbopyricularia*, and *Nigrosynnema*. A new combination, *Nigrosynnema natarajanensis*, is proposed for the previously described *Virgatospora natarajanensis*. In this study, we found several species, *Bussabanomyces wurfbainiae*, *Corynespora cassiicola*, *Curvularia geniculate*, *Musicillium tropicale*, *Muyocopron laterale*, *Musidium wurfbainiae*, *Sarocladium wurfbainiae*, in all three life forms, namely endophytic, saprobic, and pathogenic, from

Wurfbainia villosa. We also discovered numerous fungi that were restricted to specific plant tissues such as leaves, stems, roots, or fruits. However, some fungi, including *Bulbopyricularia guangdongensis*, *Corynespora wurfbainiae*, and *Musidium wurfbainiae*, were reported from multiple plant tissue types, occurring across all examined tissues. Hence, we suggest that the lifestyle of the same fungus within a plant may shift rather than remaining fixed. Meantime, we also observed that some endophytic fungal species produce appressoria, the specialized infection structures typical of pathogens, such as *Colletotrichum*, *Fusarium*, and *Bulbopyricularia*. This strongly supports the possibility that endophytes may shift to a pathogen lifestyle. Furthermore, we provided a global checklist of fungi associated with *Amomum villosum*, revealing that most species have been reported from China, with endophytic fungi representing the majority.

Furthermore, we evaluated and compared three isolation methods, plant tissue isolation, tissue maceration, and intercellular fluid culture, for their effectiveness in isolating endophytic fungi from various tissues of *Wurfbainia villosa*. Our results demonstrated that plant tissue isolation is the most effective method for recovering endophytic fungi across all tissue types. However, we are not sure whether tissue maceration showed potential for isolating slow-growing fungi, and intercellular fluid culture is suitable for root endophyte isolation of *Wurfbainia villosa*. We further tested a range of sporulation culture media and identified that plant tissue + pine needle-enriched medium and fruit juice medium yielded the highest sporulation rates. These findings offer valuable methodological insights for the effective isolation and cultivation of endophytic fungi from *W. villosa*.

In addition to the experimental results, we provide a comprehensive review of the diversity and ecological roles of endophytic fungi, and propose a refined definition that incorporates their colonization behavior, potential pathogenicity, host benefits, and lifestyle diversity. The integrative approach combining morphological analysis and multi-gene phylogenetic study has led to more accurate taxonomic placements, significantly enhancing our understanding of fungal diversity associated with *Wurfbainia villosa* in southern China, an area and host that have been largely understudied. This study not only reveals the rich fungal biodiversity linked to *W. villosa* but also provides insights into fungal lifestyle changes. Furthermore, the fungal

specimens collected have been deposited in herbarium and culture collections, serving as valuable resources for future research in fungal taxonomy and the exploration of their biomaterial properties.

Keywords: Ascomycota, Dothideomycetes, Sordariomycetes, Fungal Lifestyle Change, Chunsharen Plant, Zingiberaceae, Taxonomy, Multi-locus Phylogeny

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