Mycological Explorations of Southern United States: a Journey of Discoveries. Part 1

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A summary of taxonomic and phylogenetic studies of saprobic microfungi collected in southern United States is presented. One novel genus and two species of asexual Ascomycota were described new to science from the southwestern states of Arizona and New Mexico.

Ascomycota is the largest phylum of the kingdom Fungi. Most of its members are filamentous and many of them are capable of reproducing both sexually or asexually. However, a large number of ascomycetes are known only by their asexual states and they are called anamorphic, mitosporic, conidial or asexual fungi. Their most common and morphologically diverse type of asexual propagule is the conidium, which is produced by mitosis on specialized conidiogenous cells born on modified hyphae named conidiophores and easily dispersed by wind and water splash. Ecologically, these fungi are often saprotrophs able to colonize a wide range of substrates and habitats in nature, actively participating in the decomposition and recycling of nutrients in ecosystems while releasing secondary metabolites and bioactive compounds with many different biotechnological, industrial and pharmacological applications. The systematic position of many of these asexual fungi is unknown due to the limitations of morphological and developmental characters to establish reliable phylogenetic relationships. DNA sequence data in combination with methods of statistical inference are used nowadays to incorporate them into the current classification of the Fungi.

On the other hand, the southern states of the United States, from California to the west to Florida in the southeast, include a wide spectrum of ecological regions encompassing tropical wet and eastern temperate forests, plains, deserts and forested mountains, as well as a variety of climates ranging from arid or semi-arid to humid subtropical and Mediterranean. The term 'southern' is not used here in a historical or geographical sense but applies to those states along the southern border or lining the Gulf of Mexico. Such a large and ecologically diverse region is expected to harbor a wide range of novel microscopic fungi belonging to the asexual Ascomycota, particularly those inhabiting dead plant debris and having a saprobic lifestyle.

The diversity of this group of microfungi has been systematically studied during the past nineteen years in collaboration with mycologists from Czech Republic, Germany, Japan, Mexico, Spain and United States. Several fieldworks were carried out in forested or suburban areas of Arizona, California, Florida and Texas with the aim to collect pieces of dead wood, branches, twigs, decaying bamboo culms, palm rachides, petioles, inflorescences, climbing vines and leaf litter among other substrates usually colonized by these microorganisms. Colonies were detected in the field using a hand lens and materials were brought to the laboratory for further processing. They were first washed-off under tap water and incubated in moist chambers at room temperature (23–25°C) to enhance growth and sporulation followed by periodical examinations under the stereomicroscope. Fungal structures were studied and measured under the light microscope (LM) at 1000× magnification. To obtain cultures, single-spore isolations were performed by picking-up conidia with a sterile needle and placing them aseptically on malt extract agar (MEA) or potato dextrose agar plates. For scanning electron microscopy (SEM), pieces of natural substrate or culture media with colonies were processed following standard sample preparation protocols. Voucher specimens and strains are deposited in international herbaria and culture collections. Genomic DNA was extracted from cultures using different protocols followed by PCR amplification and DNA sequencing of nuclear ribosomal and protein coding gene markers used nowadays in fungal phylogenetics studies. Methods of phylogenetic reconstruction such as Maximum Likelihood or Bayesian Inference were employed

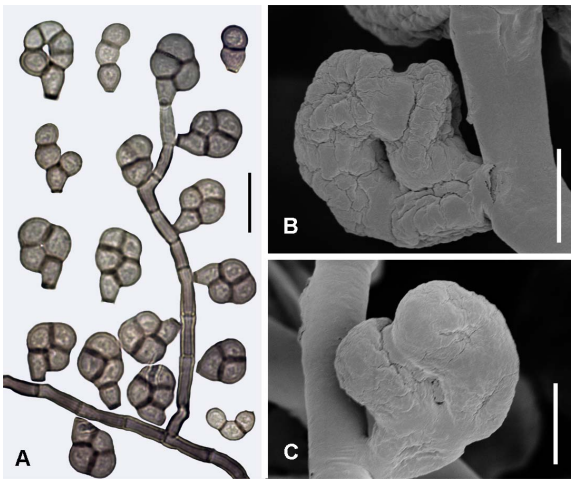

Figure 1: *Curviclavula anemophila*. Conidiophores, conidiogenous cells and conidia in LM (A) and SEM (B–C). Scale bars: A = 10 µm; B, C = 3 µm. Source [1]

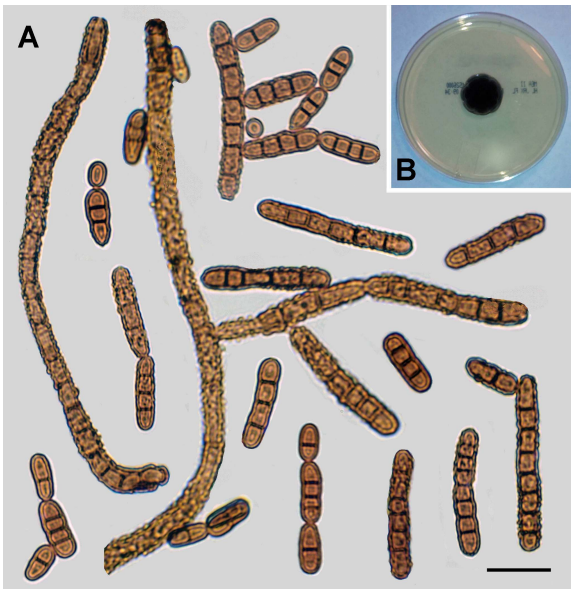

Figure 2: *Septonema lomanii*. Conidiophores, branches and conidia in LM (A) and (B) Colony on MEA. Scale bar: A = 20 µm; B = 1 cm. Source [2]

to infer relationships from molecular data and to estimate phylogenetic trees. Novel DNA sequences were publicly deposited in GenBank.

Despite being popularly depicted as desertic or arid states, Arizona and New Mexico both contain in the north large tracts of montane coniferous forest dominated by the widespread Ponderosa pine among other plant substrates suitable for the development of a rich saprobic mycobiota. Mycologically, however, microfungi have been relatively poorly explored compared with mushrooms or lichenized fungi. As a result of studying samples from these areas, distinct morphological, cultural and molecular characters led to the description of one novel genus, *Curviclavula*, and two new species named *C. anemophila* and *Septonema lohmanii*.

In the case of *Curviclavula*, the source colony was recovered from an air sample collected outdoors in northern New Mexico [1]. The fungus took its name from the peculiar club shape and unique development of its conidia (Figure 1). They resemble at first a tiny hand in side view, but the apical cells of the developing conidium later curves and fuses with the lateral and adjacent cells once they become in contact eventually forming a tightly appressed, subglobose to broadly club-like shaped conidium. Phylogenetic analyses of molecular data revealed a placement within the order Helotiales in the class Leotiomycetes and affinities for members of the family Hyaloscyphaceae.

Septonema lomanii, on the other hand, was collected independently in both northern Arizona and the Czech Republic on two different pine species [2]. The fungus is distinct among *Septonema species* by its heavily ornamented conidiophores, branches, conidia and hyphae, ranging from verruculose to strongly verrucose with prominent rounded warts and yellowish brown to brown or reddish brown in color (Figure 2). Multigene phylogenetic analyses suggested a placement within the Mytilinidiales belonging to the class Dothideomycetes and the first time a septonema-like fungus is linked to the order using molecular data. Based on morphological and molecular evidence, both the Arizona and Czech collections were considered conspecific despite their disjunct geographical distribution.

Future explorations will continue uncovering the outstanding diversity of microfungi in southern United States especially in poorly studied areas of the southwest with a variety of habitats and ecosystems.

Notes

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References

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[2] Delgado, G., Koukol, O., Miller, A. N. and Piepenbring, M., *Septonema lohmanii*, a new species in Mytilinidiales (Dothideomycetes) and the phylogenetic position of *S. fasciculare*, *Cryptogamie Mycologie* **40** (2019) 3–21