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Leohumicola, a genus new to China

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Abstract —The anamorph genus *Leohumicola* is newly recorded from China. *Leohumicola verrucosa* was isolated from the root of a pharmaceutical plant, *Pleione yunnanensis* (*Orchidaceae*), collected in Guizhou Province. The morphology of this species is described and illustrated.

Key words — hyphomycetes, leotiomycetes

Introduction

The hyphomycetous genus *Leohumicola* was established by Hambleton et al. (2005) with four species (*L. verrucosa, L. minima, L. terminalis,* and *L. lenta*), with *L. verrucosa* as the type. Nguyen & Seifert (2008) added three new species (*L. levissima*, *L. atra* and *L. incrustata*) from South Africa and the United States and assessed the utility of both the ribosomal internal transcribed spacer (ITS) and cytochrome oxidase 1 (*Cox1*) as DNA barcodes for the identification of *Leohumicola* species. Species of the genus produce two-celled conidia, with a round to ellipsoidal, dark-brown, terminal cell that is either smooth or warty, and a basal cell that is either cupulate or cylindrical, and hyaline to pale brown. Most *Leohumicola* strains grow slowly on potato-dextrose agar (PDA) and tend to produce sterile colonies, often surrounded by yellow, green brown, or red pigments.

Several of the known species of *Leohumicola* are associated with burnt ecosystems, especially commercial blueberry cultivation, and were isolated by heat treatment of soil suspensions or from surface-sterilized roots of ericaceous host plants (Nguyen & Seifert 2008). Recently, we isolated and identified *L. verrucosa* in the course of surveys of endophytic fungi associated with a pharmaceutical plant in the family *Orchidaceae*. This fungal genus has not

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been reported in China previously. An illustrated description of the species is provided in the present paper based on our isolate from China.

Materials and methods

Healthy plants of *Pleione yunnanensis* Rolfe were collected from the Guizhou Province, China. The isolation of endophytic fungi was performed by the method described by Hu (2007). Colony and microscope descriptions are based on cultures grown on PDA at 25 °C under ambient light conditions in the laboratory. Measurements and microscopic photographs were taken from material mounted in 5 % KOH using a digital microscope camera on a ZEISS Axio Imager AI compound microscope with differential interference contrast (DIC) optics and captured using ImagePro Plus 6.0. The isolate was preserved as a living culture in the Center for Culture Collection of Pharmaceutical Microorganisms (CPCC, http://cpcc.org.cn/).

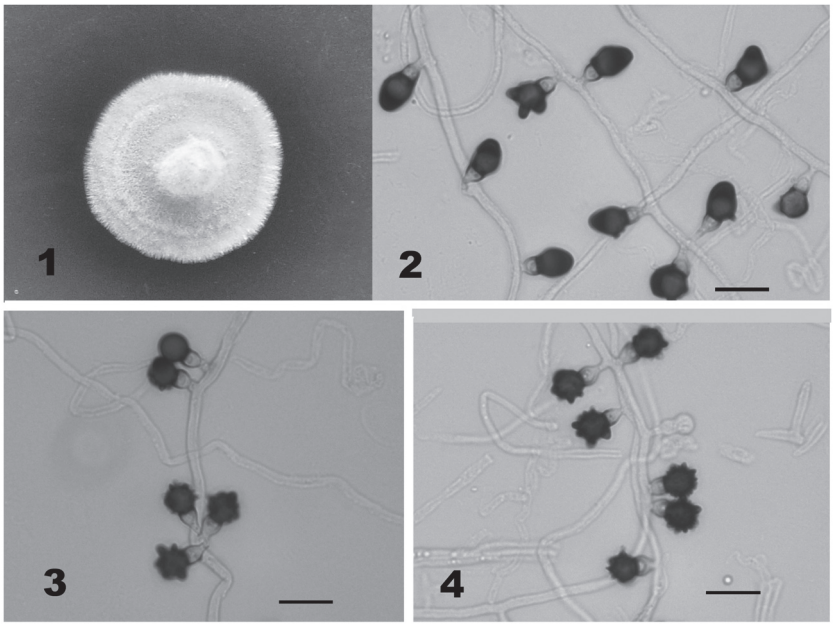
Taxonomy

Leohumicola verrucosa N.L. Nick., Hambl. & Seifert, Studies in Mycology 53: 44, 2005. Figs.1–4.

Colonies on PDA after 2 weeks under ambient light at room temperature (Fig.1) 10 mm diam, greyish green to olive yellow, or in the absence of yellow pigments, white to grey, with concentric rings, sometimes darkest near the inoculum, usually with more or less white margin, planar or convex, with low, felty, slightly lanose, or radiating funiculose aerial mycelium. Exudates not produced, pigment reddish brown. Margin smooth and entire. Colony reverse dark brown when soluble pigments are produced.

Conidiogenous hyphae hyaline, 1.5–2 µm wide. Conidiogenous cells reduced to a single denticle 1–3.5 µm long and 1–2 µm wide, the denticles single or the cell proliferating sympodially up to four times to produce a node or elongated cluster of divergent denticles (Fig.3).

Conidia (Figs.2–4) initially two-celled, single or side by side in small clusters, or with up to four successively produced sympodially from hyphae. Terminal cell 4.5–5.2(6.2) × 4–5 µm excluding the ornamentation, globose, subglobose to ovate, at first the same colour as the basal cell, then becoming dark brown while still attached, with walls slightly thickened, usually verrucose or echinulate; older conidia sometimes remaining smooth in some strains but more often with finger- or bubble-like projections or spines(Fig.2). Ornamentation 1–2 µm long, 0.7–2 µm wide at the base. Conidial connection to basal cell 2–3 µm wide, often constricted; basal cell 2.7–3.2 µm, cupulate, symmetrical or asymmetrical, subhyaline to pale brown; ratio of lengths of terminal: basal cell 1.4–2.1 µm.



Figs. 1–4. *Leohumicola verrucosa* (CPCC810187)
1. Colonies of *L. verrucosa* growing on PDA, after 14 d at room temperature;
2–4. Showing conidiogenous hyphae, conidia, and ornamentation of the terminal cell.
Scale bar =10 μm.

Chlamydospores sparsely produced. Vegetative mycelium often with swollen, hyaline or subhyaline hyphae 3–8 μm wide, constricted at septa, with slightly thickened walls.

Substrate and distribution: soil, roots; Canada, Puerto Rico, China.

Specimens examined: **CHINA**: GUIZHOU PROVINCE, Leigong County, Leigong Mountain, from root of *Pleione yunnanensis* Rolfe, 28 June 2007, Zhi-Xia Meng 3231(CPCC810187).

Remarks: The conidia of *L. verrucosa* are most similar to those of *L. incrustata* H.D.T. Nguyen & Seifert, but in the latter species, the walls are ornamented with larger, amorphous warts. Morphological features of the Chinese isolate are in agreement with the description by Hambleton et al. (2005), with a slight difference in colony morphology. The colony of the Chinese culture lacks the **conspicuous** sectoring in PDA that characterizes the original description. Our molecular analysis of the ITS nuclear rDNA region (FJ224102) supports the conspecificity of the Canadian and Chinese isolates (100% similarity GenBank sequence from *Leohumicola verrucosa*, AY706322).

Leohumicola verrucosa was originally described from Canada and Puerto Rico. Most isolates were from heated soil from coniferous forests and commercial low bush blueberry fields, or roots of *Ericaceae* and *Pinaceae*. In this paper, it was isolated from roots and pseudobulbs of *Pleione yunnanensis* (*Orchidaceae*) in China. Our collection considerably extends the host plant association and distribution for *L. verrucosa*.

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