PHYLOGENY AND SPECIES DELIMITATION OF NORTH AMERICAN LECCINOID FUNGI

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Abstract

Leccinoid fungi include ectomycorrhizal members of the Boletales (Basidiomycota, Agaricomycetes) with scabrous to pseudo-scabrous stipes and non-ornamented basidiospores, commonly collected in summer and fall in diverse forest types throughout North America. Previous phylogenetic work on leccinoid fungi has focused primarily on European species; this project centers around North American species. Using collections made by the authors and herbarium material, the initial focus of the research will be the oak-associated leccinoid fungi, which have traditionally been placed in *Leccinum*, *Boletus*, and *Leccinellum*. Sequences from two nuclear ribosomal DNA regions (LSU and ITS) and from one protein-coding gene (TEF1-alpha) have been amplified, sequenced, and analyzed in combination with morphological and ecological data to resolve the continent's major species groups. The working hypothesis for the research, suggested by previous molecular studies, is that ectomycorrhizal host specificity roughly correlates to major clades among the leccinoid fungi, and that species groups can be further delineated with a combination of molecular and morphological characters. Preliminary results of the ongoing research based on LSU and TEF data are presented.

Discussion/Conclusions

This study presents preliminary results from ongoing research into the leccinoid fungi; only about 20 species of the approximately 134 species of *Leccinum/Leccinellum* that have been described were included. Therefore, further work is needed, including additional taxon sampling and multilocus analyses, to determine species delimitations among the leccinoid fungi of North and Central America.

Our results suggest that the genus *Leccinum* needs to be revised. LSU and TEF molecular data indicate that species of *Leccinum* sections *Leccinum*, *Scabra* and *Luteoscabra*, along with *Boletus longicurvipes*, are related, and that their placement within the same genus should be considered; these species also appear closely related to species of *Rossbeevera*, *Octaviania* and *Chamonixia*.

Leccinum subglabripes does not belong to the genus *Leccinum*, but it is related to the *Xerocomus subtomentosus* group. Furthermore, *Boletus rubropunctus* and *Boletus hortonii* are not closely related to *Leccinum* species but to the *Xerocomus subtomentosus* group, as suggested in previous studies.

Our results also suggest the possibility that some GenBank sequences for leccinoid fungi may represent misidentifications.

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Materials and Methods

A total of 145 LSU and 82 TEF1 sequences were used in the present study; of these 61 LSU and 53 TEF1 were newly generated from herbarium specimens obtained from the Center for Forest Mycology Research (CFMR, Madison, WI), the Stover-Ebinger Herbarium (EIU, Charleston, IL), the New York Botanical Garden (NY, Bronx, NY), the U. S. National Fungus Collections (BPI, Beltsville, MD) and the personal herbarium of Michael Kuo (Charleston, IL), while 82 LSU and 29 TEF1 were retrieved from GenBank. GenBank sequences primarily represent isolates of material from Europe and Asia, while the newly generated sequences are from the United States and Belize.

The 5' end of the LSU region was amplified with primer pair LROR/LR5, and TEF1 was amplified using primer pair EF1-983/EF1-1567R. Sequences were edited with Sequencher 4.8 and aligned using MAFFT v.6. The alignments were manually adjusted using MacClade 4.08.

The phylogenetic relationships among leccinoid species were evaluated with two DNA sequence datasets (LSU and TEF) and maximum likelihood (ML) analyses. ML was run in the RAxML server, v.7.2.8, under a GTR model with 100 rapid bootstrap replicates. The LSU dataset included sequences of about 19 species of *Leccinum/Leccinellum* and at least one species of the *Boletus*, *Chamonixia*, *Harrya*, *Octaviania*, *Roosbeevera*, *Sutorius* and *Xerocomus*, and the TEF dataset included about 15 species of *Leccinum/Leccinellum* and at least one of *Boletus*, *Harrya*, *Octaviania*, *Sutorius* and *Xerocomus*.

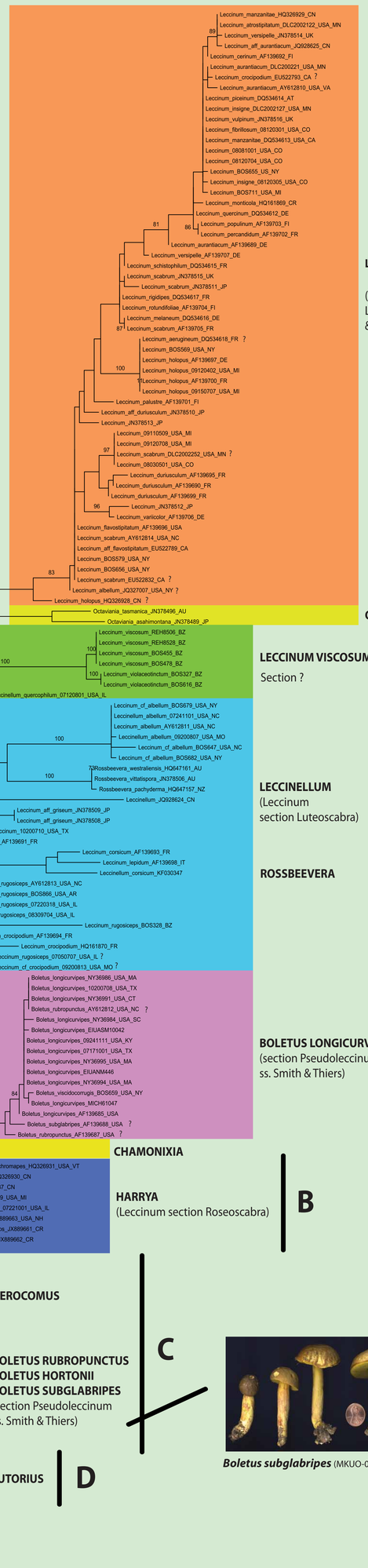
Results

In the analysis of the LSU dataset (**FIG 1**), leccinoid fungi were grouped into four moderately to strongly supported main clades: the **Leccinum (A)**, **Harrya (B)**, **Xerocomus (C)** and **Sutorius (D)** clades.

Within the **Leccinum clade** six groups were obtained: the **Leccinum group** (species of *Leccinum* sections *Leccinum* and *Scabra*), the **Octaviania group** (*Octaviania* species), the **Leccinum viscosum group** (species of *L. viscosum*, *L. violaceotinctum* and *L. quercophillum*), the **Leccinellum group** (species of *Leccinellum* and *Rossbeevera*), the **Boletus longicurvipes group** (species of *Boletus* section *Pseudoleccinum* ss. Smith & Thiers) and the **Chamonixia group** (*Chamonixia caespitosa*); the Leccinum viscosum and Leccinellum groups were not statistically supported.

The **Harrya group** includes *H. chromapes* (previously *Leccinum* section *Roseoscabra*) and *H. atriceps*. The **Xerocomus clade** includes species of *Xerocomus* (section *Xerocomus*), *Boletus hortonii*, *B. rubropunctus* and *Boletus subglabripes* (*Boletus* section *Pseudoleccinum* ss. Smith & Thiers). The **Sutorius clade** includes *S. australiensis* and *S. eximius* (previously *Leccinum* sect. *Eximia*). In the analysis of the TEF dataset (**FIG 2**) these four main clades were also recovered and the Leccinum viscosum and Leccinellum groups were also not statistically supported.

Figure 1. Phylogeny based on the nLSU dataset.



***Leccinum fibrillosum*/ *Leccinum vulpinum*/ *Leccinum insigne* ?**

Leccinum fibrillosum (MKUO 08120301)

***Leccinum* sp.** (MKUO 08120704)

***Leccinum* sp.** (BOS-711)

Leccinum holopus
(MKUO 09120402)

***Leccinum* sp.** (MKUO 09120708)

***Leccinum* sp.** (BOS-656)

Leccinum viscosum
(REH-8528)

Leccinum violaceotinctum (BOS-327)

Leccinellum quercophilum
(MKUO 07120801)

Boletus longicurvipes (BOS-659)

Harrya chromapes (MKUO 09150709)

Leccinum* cf. *albellum (BOS-582)

Leccinum* cf. *rugosiceps (BOS-328)

Leccinum crocipodium ?
(MKUO070507

Boletus subglabripes (MKUO-08240506)

Figure 2. Phylogeny based on the TEF dataset.