

# Genetic Diversity within Alaskan *Boletus* Mushrooms



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## Abstract

We analyzed the genetic differences within the collection of *Boletus* mushroom from the UAF Fungal Herbarium, representing samples from all over Alaska. Upon analyzing the DNA sequence from 18 samples, we found that most Alaskan *Boletus* are closely related to samples from the same species found in other parts of the world (clades 1,2,5,6,8,12). We also found that some species collected in other parts of the world were not found in our sample collection (clades 4,7,9,10,11). Finally, our results suggested that clade 3 on our phylogenetic tree is not represented among the GenBank sequences from other parts of the world and may be a genetic lineage endemic to Alaska.

## Introduction

In this study we used DNA to distinguish the genetic affiliations within *Boletus* species and possibly identify new species within this genus in Alaska. Boletes are basidiomycete mushrooms that have a spongy layer of tubes on the bottom of their cap instead of gills. This layer in the boletes can peel away from the cap. There are many genera among the boletes including *Suillus*, *Leccinum* and *Tylopilus*, but the species in genus *Boletus* are some of the most well-known and prized mushrooms in the world. Boletes are mycorrhizal. Boletes will stain blue or greenish blue when bruised. The boletes' stalk is usually covered with veins. The spore color in boletes range from yellow to olive, brown, reddish brown, chocolate-brown or black. Boletes are mostly edible with only a few boletes that are poisonous. The geographical range for *boletus* is world wide and many occur frequently in North America (Arora). Which of the species exist in Alaska had not been determined using genetic methods before this study.

## Materials and Methods

We extracted DNA using the SP Fungal Mini Prep Protocol. The changes to this protocol were minimal. First we ground the mushroom samples with two 3.2mm stainless beads in a ball mill. After that we then ran a gel to see if we had obtained any DNA product from our samples. The extraction was successful as seen in the figure below. We then went forward and did PCR for all of the samples, the results also showed success, as seen in figure 2 below. After that we did a PCR clean up using the standard protocol without any changes. Then we submitted the samples to the UAF DNA Core Lab and got the sequences for them, assembled reads in Sequencher, used ClustalW to build a sequence alignment. We then built a phylogenetic tree using the Maximum Likelihood algorithm in the program GARLI after we downloaded representative GenBank sequences to compare them with the sequence from the sample DNA we had extracted.

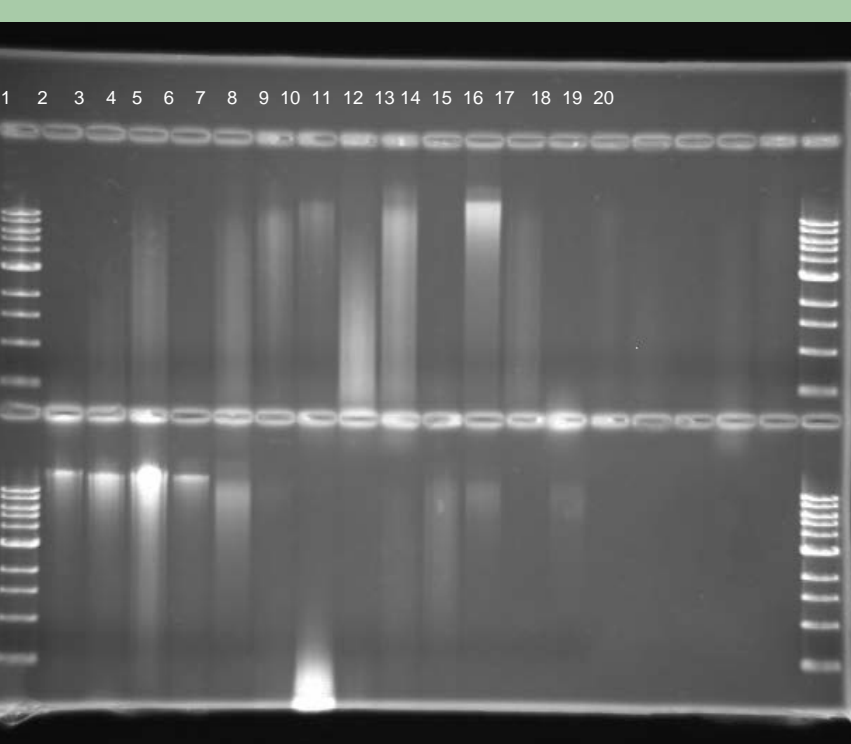


Figure 1. The DNA gel run with all of the samples labeled with the numbers 1-20. Samples 1 and 20 are DNA ladder.

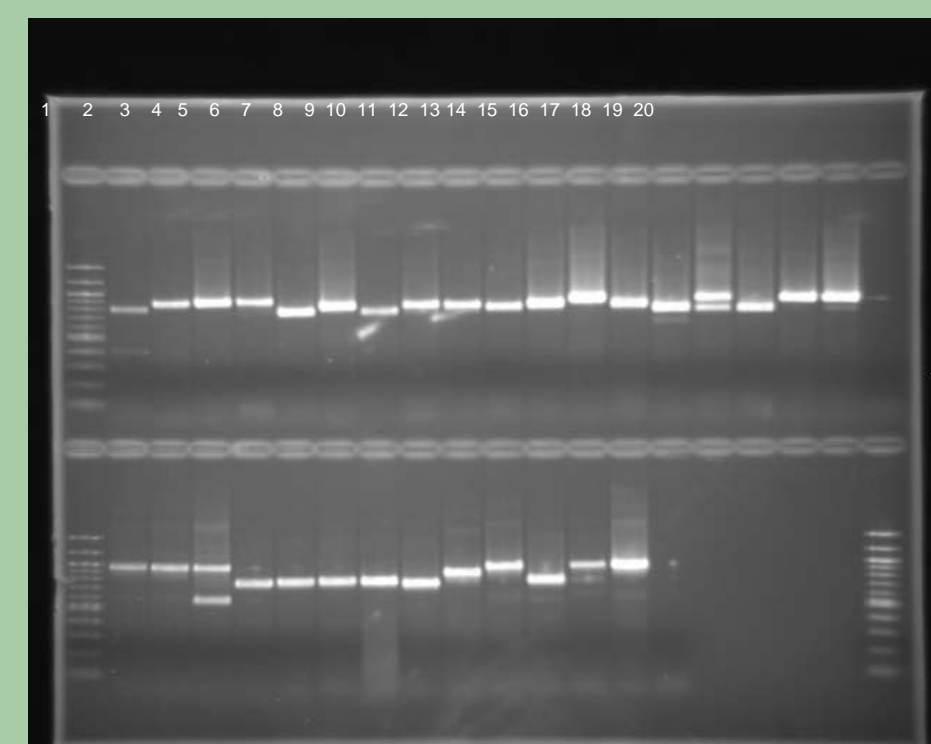


Figure 2. the PCR gel run with all of the samples labeled with numbers 1-19. Sample 1 is our ladder.

## Results

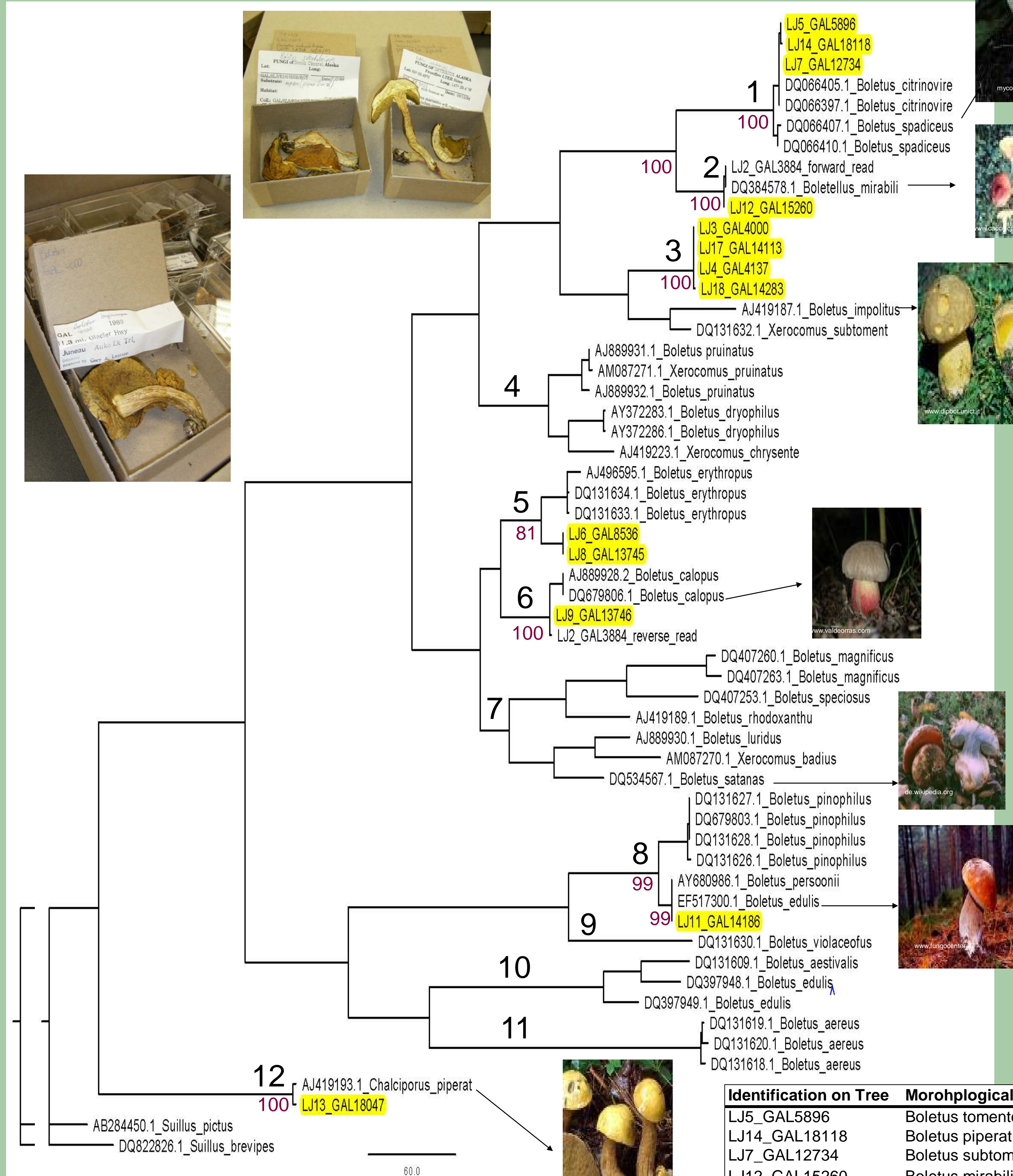


Figure 1 (Above). Maximum Likelihood tree of Alaskan samples (highlighted) compared with the GenBank sequences. Clades are numbered at the "nodes," and numbers below nodes are the bootstrap support values (out of 100).

Identification on Tree	Location
DQ066405_Boletus_citrinovirens	Europe
DQ066407_Boletus_spadiceus	Europe
DQ066397_Boletus_citrinovirens	Europe
DQ066410_Boletus_spadiceus	Europe
DQ384578_Boletellus_mirabilis	Europe
AJ419187_Boletus_impolitus	Spain
DQ131632_Xerocomus_subtomentosus	Europe
AJ889931_Boletus_pruinatus	Europe
AM087271_Xerocomus_pruinatus	Europe
AJ889932_Boletus_pruinatus	Europe
AY372283_Boletus_dryophilus	CA, U.S.A.
AY372286_Boletus_dryophilus	CA, U.S.A.
AJ419223_Xerocomus_chryseron	Spain
AJ496595_Boletus_erythropus	Spain
DQ131634_Boletus_erythropus	Europe
DQ679806Boletus_calopus	Europe
DQ407260_Boletus_magnificus	China
DQ407263_Boletus_magnificus	China
DQ407253_Boletus_speciosus	China
AJ419189_Boletus_rhodoxanthus	Spain
AJ889930_Boletus_luridus	Europe
AM087270_Xerocomus_badius	Europe
DQ534567_Boletus_satanas	Ma. U.S.A.
DQ131627_Boletus_pinophilus	Europe
DQ679803_Boletus_pinophilus	Europe
DQ131628_Boletus_pinophilus	Europe
DQ131626_Boletus_pinophilus	Europe
AY680986_Boletus_persoanii	Europe
EF517300_Boletus_edulis	Czech Rep.
DQ131630_Boletus_violaceofuscus	Europe
DQ131609_Boletus_aestivalis	Europe
DQ397948_Boletus_edulis	China
DQ397949_Boletus_edulis	China
DQ131619_Boletus_aereus	Europe
DQ131620_Boletus_aereus	Europe
DQ131618_Boletus_aereus	Europe
AJ419193_Chalciporus_piperatus	Spain
AB284450_Suillus_pictus	Japan
DQ822826_Suillus_brevipes	CA, U.S.A.

Figure 2. Table of GenBank sequences.

Identification on Tree	Morphological ID	GenBank Affiliations	Clade	Collection Date	Location
LJ5_GAL5896	Boletus tomentosus	Boletus citrinovirens*	1	8.07.1994	NorthWest, AK
LJ14_GAL18118	Boletus piperatus	Boletus citrinovirens*	1	7.23.2005	Interior AK
LJ7_GAL12734	Boletus subtomentosus	Boletus citrinovirens*	1	8.15.2000	Kobuk, AK
LJ12_GAL15260	Boletus mirabilis	Boletus mirabilis	2	9.02.2001	SouthEast, AK
LJ2_GAL3884	Boletus mirabilis	Boletus mirabilis	2	8.11.1989	SouthEast, AK
LJ3_GAL4000	Boletus longicurrupes	no close ITS matches	3	1989	SouthEast, AK
LJ17_GAL14113	Boletus subglabripes	no close ITS matches	3	7.13.1998	SouthCentral, AK
LJ4_GAL4137	Boletus subglabripes	no close ITS matches	3	7.25.1990	SouthEast, AK
LJ8_GAL14283	Boletus subglabripes	no close ITS matches	3	8.13.1998	Interior, AK
LJ8_GAL8536	Boletus erythropus	Boletus erythropus	5	7.29.1998	SouthCentral, AK
LJ6_GAL13745	Boletus erythropus	Boletus erythropus	5	7.30.1992	Interior AK
LJ9_GAL13746	Boletus coniferarum	B. coniferarum and B. calopus	6	7.30.1992	Interior AK
LJ11_GAL14186	Boletus edulis	Boletus edulis	11		NorthWest, AK
LJ13_GAL18047	Boletus piperatus	Chalciporus piperatus	12	7.21.2005	AK

Figure 3. Table of Alaskan sequences generated in this study.



Figure 4. Photo of Dr. Larsen's herbarium where we obtained the Alaskan samples from.



Figure 6. A photo of *B. campestris*

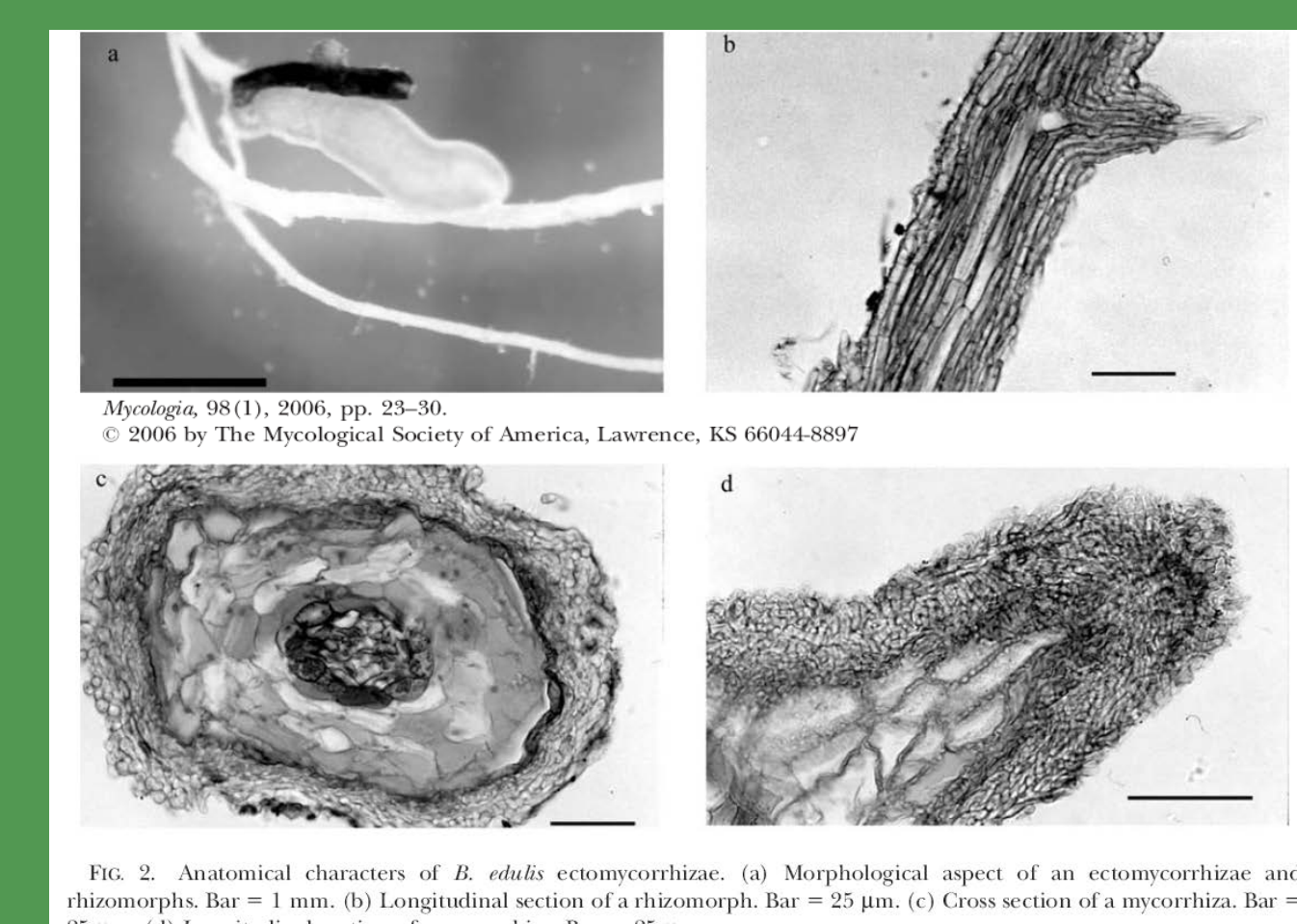


Figure 7. A mycorrhizal root (from previous study)

## Discussion

• **Clade 1** is composed of Alaskan samples from the interior and Northwest and samples from Europe. Although the species names do not match up they are genetically very similar, and *B. citrinovirens* is thought to be in what is called the *subtomentosus* group.

• **Clade 2** is composed of Alaskan samples from Southeast Alaska and GenBank samples from Europe, both identified as *Boletus mirabilis*.

• **Clade 3** is composed of samples only from Alaska. None of the sequences obtained from GenBank are closely related. These were morphologically identified as *B. subglabripes*. "*B. subglabripes*" is in GenBank, but only Large Subunit gene, not Internally Transcribed Spacer gene (reference 2) so comparison is not possible to determine if morphological species attribution is the same or not.

• **Clade 4** is a group of GenBank sequences (*B. pruinatus*, *B. dryophilus* and *X. chryseron*) that did not show significant amounts of similarity to the samples I extracted. This appears to be a group that is found in Europe and California, but has not been sampled in Alaska.

• **Clade 5** is closely related to *B. erythropus* with my sequences LJ6\_GAL8536 and LJ8\_GAL13745, and represents a species found in Europe and Southcentral and interior Alaska.

• **Clade 6** are closely related to *B. calopus* from Europe with my sequence LJ9\_13746 from interior Alaska.

• **Clade 7** are the GenBank sequences (*B. magnificus*, *B. speciosus*, *B. rhodoxanthus*, *B. luridus*, *X. badius* and *B. satanas*) that did not show significant amounts of similarity to Alaskan samples.

• **Clade 8** is composed of GenBank sequences attributed to *B. persoanii* and *B. pinophilus* are closely matched up with my sequence LJ11\_GAL4186 however, that sequence most closely grouped up with *B. edulis* from Northwest Alaska. This is the famous King Bolete.

• **Clade 9** is composed of GenBank sequences *B. violaceofuscus* which did not show significant amounts of similarity to any Alaskan samples.

• **Clade 10** are the GenBank sequences *B. aestivalis* that did not show significant amounts of similarity to the samples I extracted. There are two sequences attributed to *B. edulis* among the GenBank sequences that are probably misidentifications.

• **Clade 11** are the GenBank sequences for *B. aereus* that did not show significant amounts of similarity to the Alaskan samples.

• **Clade 12** is composed of the Genbank sequence AJ419193\_ *Chalciporus piperatus* and my sample LJ13\_GAL18047 which was originally identified as *B. piperatus* mean the exact same thing. *Chalciporus* was the first naming of the genus and *Boletus* is the recent naming.

## Bibliography

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