GENETIC DIVERSITY WITHIN ALASKAN MORELS

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ABSTRACT

We isolated DNA from 12 "true morels" (*Morchella*) and one "false morel" (*Gyromitra*) collected in interior Alaska. We PCR amplified the Internally Transcribed Spacer region of the ribosomal DNA genes. After cycle sequencing our amplified gene, we used the Sequencher program to assemble the raw sequences, ClustalW to align sequences across samples, and the GARLI program to create a phylogenetic tree of evolutionary history that compares our samples with all publicly available *Morchella* sequences, obtained from GenBank. We also included sequences obtained directly from interior AK forest soils and found close matches to aboveground morels. In general we found that our Alaskan samples span much of the diversity of various groups of "black" morels, but we found no "yellow" morels. Interestingly, five of our samples from 3 interior AK burn sites composed a group that appears distinctly Alaskan.

INTRODUCTION

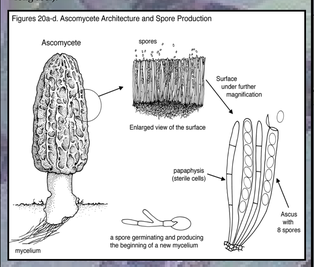
DESCRIPTION AND DIVERSITY

Morels are cone-shaped Ascomycete mushrooms often found growing prolifically in burned soil the summer after a wildfire and occasionally in other disturbed habitats. All morels are choice edibles and easily identifiable as a genus. However, morels exhibit a wide variety of forms and colors, and individual species are difficult to identify. Current estimates put North America with 4 "yellow" and 9 "black" morels (Pilz et. al.), although we provided evidence that an entirely separate group exists (see figure 1).

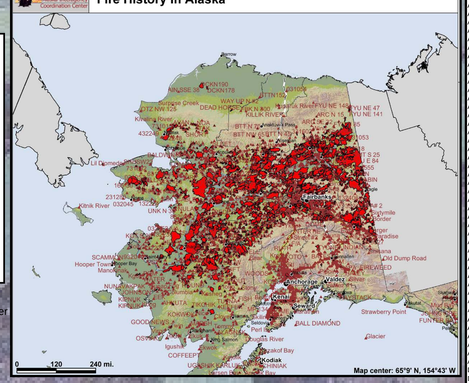
BIOLOGY

Morels are the sporocarps (fruiting bodies) of underground mycelia, the microscopic fungal threads that spread through the soil and collect water and nutrients. It is poorly understood whether morels are symbionts (organisms that live in a mutually beneficial relationship with other organisms), saprophytes (organisms that decompose decaying matter), or parasites (organisms that feed directly off of other living organisms). It seems likely that across the various species or across the lifespan within a species all three lifestyles may occur. The morel sporocarp itself grows only for about 1-6 days before releasing spores and disintegrating. The spores float in the wind, some eventually landing on an area suitable for germination. Some theories suggest that the spores germinate soon after hitting the soil and remain as mycelia until a fire or other disturbance. Others imply that the spores remain underground for long periods, then germinate after a fire and produce sporocarps the following summer (Pilz et. al.)

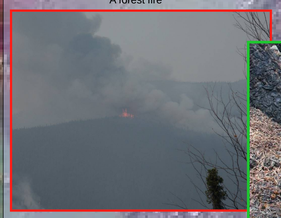
The flush of organic matter from the roots of dead plants may provide the resources necessary for sporocarp production. However, it has also been suggested that the mycelia produce sporocarps for entirely different reasons—that the burned environment is too harsh for the mycelia, which put their last resources into reproduction before they die. The importance of morels in the ecosystem, especially in replenishing nutrients to burned soil, is unknown (Pilz et. al.).



(Below) The life cycle of a morel. Morels are Ascomycetes (mushrooms that contain their spores in long asci).

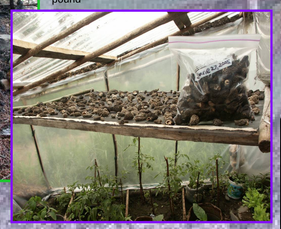


(Left) A map of total burn area since 1960. More land burns every year in Alaska than in all the other states combined, and yet most wildfires occur in such remote areas that only a tiny fraction of the resulting morels are harvested.



A forest fire

Morels grow in the burned soil the next year



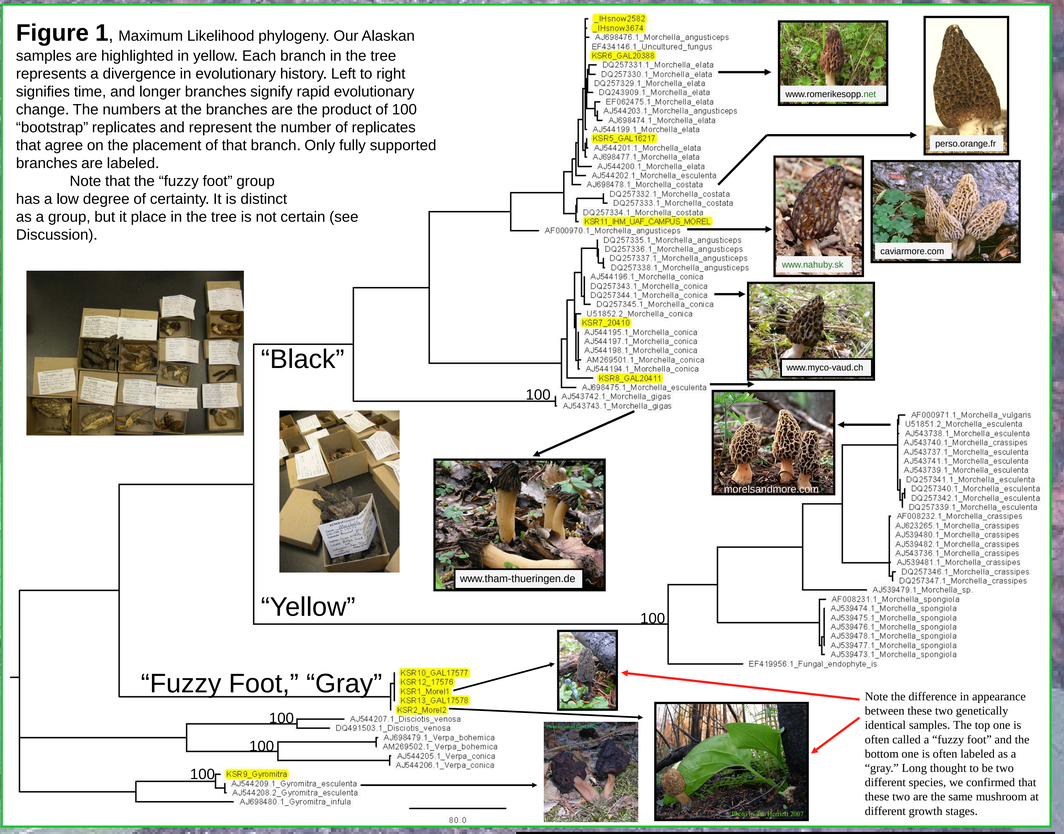
They are gathered, dried, and sold at about $65 per pound

The Morel Crop

Morels are gathered in large quantities where they occur, and result in a sale of $5 million to $10 million annually in Western North America (Pilz et. al.).

Figure 1, Maximum Likelihood phylogeny. Our Alaskan samples are highlighted in yellow. Each branch in the tree represents a divergence in evolutionary history. Left to right signifies time, and longer branches signify rapid evolutionary change. The numbers at the branches are the product of 100 "bootstrap" replicates and represent the number of replicates that agree on the placement of that branch. Only fully supported branches are labeled.

Note that the "fuzzy foot" group has a low degree of certainty. It is distinct as a group, but it place in the tree is not certain (see Discussion).



Identification on Tree	Date	Geographic Location	Ref.
AF000970.1_Morchella_angustice	22.Apr.97	None given	u
AF000971.1_Morchella_vulgaris	CD 1987	Moyon, France	1
AF008231.1_Morchella_spongiola	13.Jun.97	None given	1
AF008232.1_Morchella_crassipes	13.Jun.97	None given	u
AJ539473.1_Morchella_spongiola	31.Jan.03	Thuringia:Jena:Kunitz:Kunitzburg, Germany	u
AJ539474.1_Morchella_spongiola	31.Jan.03	Thuringia:Jena:Goeschwitz, Germany	u
AJ539475.1_Morchella_spongiola	31.Jan.03	Thuringia:Jena:Goeschwitz, Germany	u
AJ539476.1_Morchella_spongiola	31.Jan.03	Thuringia:Jena:Goeschwitz, Germany	u
AJ539477.1_Morchella_spongiola	31.Jan.03	Thuringia:Eschenbergen:Fahnersche:Hoehe, Germany	u
AJ539478.1_Morchella_spongiola	31.Jan.03	Pas de Calais:Merlimont, France	u
AJ539479.1_Morchella_sp	31.Jan.03	Solan Himachal Pradesh, India	u
AJ539480.1_Morchella_crassipes	31.Jan.03	Thuringia:Jena:Drackendorf, Germany	u
AJ539481.1_Morchella_crassipes	31.Jan.03	Alsace:Illkirch-Graffenstaden, France	u
AJ539482.1_Morchella_crassipes	31.Jan.03	Saxony:Leipzig:Mark-Kleeberg, Germany	u
AJ543736.1_Morchella_crassipes	10.Feb.03	Thuringia, Jena, Drackendorf, Germany	u
AJ543737.1_Morchella_esculenta	10.Feb.03	Alsace, Offendorf, France	u
AJ543738.1_Morchella_esculenta	10.Feb.03	Jena, Grance, Germany	u
AJ543739.1_Morchella_esculenta	10.Feb.03	Baden-Wuerttemberg, Kreuzingen, Weisweil, Germany	u
AJ543740.1_Morchella_crassipes	10.Feb.03	USA	u
AJ543741.1_Morchella_esculenta	10.Feb.03	North Rhine-Westphalia, Muenster, Germany	u
AJ543742.1_Morchella_gigas	10.Feb.03	Thuringia, Jena, Kunitz, Kunitzburg, Germany	u
AJ543743.1_Morchella_gigas	10.Feb.03	Thuringia, Jena, Botanischer Garten, Germany	u
AJ544194.1_Morchella_conica	12.Feb.03	Thuringia, Jena, Kritzegraben, Germany	u
AJ544195.1_Morchella_conica	12.Feb.03	Thuringia, Jena, Kunitz, Germany	u
AJ544196.1_Morchella_conica	12.Feb.03	Thuringia, Jena, Damenviertel, Germany	u
AJ544197.1_Morchella_conica	12.Feb.03	Thuringia, Jena, Damenvierte, Germany	u
AJ544198.1_Morchella_conica	12.Feb.03	North Israel	u
AJ544199.1_Morchella_elata	12.Feb.03	Solan Himachal Pradesh, India	u
AJ544200.1_Morchella_elata	12.Feb.03	Solan Himachal Pradesh, India	u
AJ544201.1_Morchella_elata	12.Feb.03	Solan Himachal Pradesh, India	u
AJ544202.1_Morchella_esculenta	12.Feb.03	USA	u
AJ544203.1_Morchella_angustice	12.Feb.03	Canada, BC	u
AJ544205.1_Verpa_conica	12.Feb.03	Thuringia, Jena, Kunitz, Kunitzburg, Germany	u
AJ544206.1_Verpa_conica	12.Feb.03	Thuringia, Jena, Kunitz, Kunitzburg, Germany	u
AJ544207.1_Discotis_venosa	12.Feb.03	Saxony-Anhalt, Beendorf, Germany	u
AJ544208.2_Gyromitra_esculenta	30.Aug.06	Alsace, Rhinau, France	u
AJ544209.1_Gyromitra_esculenta	26.Apr.03	Liverpool, Neston, United Kingdom	2
AJ623265.1_Morchella_crassipes	29.Jan.04	Lovcen National Park, Serbia, Montenegro	2
AJ698474.1_Morchella_elata	26.Apr.04	Skomas Peninsula, Cyprus	u
AJ698475.1_Morchella_esculenta	26.Apr.04	Huatusco, Veracruz, Mexico	u
AJ698476.1_Morchella_angusticeps	19.Oct.05	Mexico	u
AJ698477.1_Morchella_elata	26.Apr.04	Huatusco, Veracru, Mexico	u
AJ698478.1_Morchella_costata	26.Apr.04	El Paraiso, Tlacco, Tlaxcala, Mexico	u
AJ698479.1_Verpa_bohemica	26.Apr.04	Bienitz, Rueckmarsdorf, Leipzig, Germany	u
AJ698480.1_Gyromitra_infula	26.Apr.04	California, Blodgett Forest, USA	u
AM269501.1_Morchella_conica	CD 2004	Zakopane, Poland	u
AM269502.1_Verpa_bohemica	CD 2004	Krakow, Poland	u
DQ243909.1_Morchella_elata	15.Oct.05	China	u
DQ257329.1_Morchella_elata	19.Oct.05	China	u
DQ257330.1_Morchella_elata	19.Oct.05	China	u
DQ257331.1_Morchella_elata	19.Oct.05	China	u
DQ257332.1_Morchella_costata	19.Oct.05	China	u
DQ257333.1_Morchella_costata	19.Oct.05	China	u
DQ257334.1_Morchella_costata	19.Oct.05	China	u
DQ257335.1_Morchella_angustice	20.Oct.05	China	u
DQ257336.1_Morchella_angustice	20.Oct.05	China	u
DQ257337.1_Morchella_angustice	20.Oct.05	China	u
DQ257338.1_Morchella_angustice	20.Oct.05	China	u
DQ257339.1_Morchella_esculenta	20.Oct.05	China	u
DQ257340.1_Morchella_esculenta	20.Oct.05	China	u
DQ257341.1_Morchella_esculenta	20.Oct.05	China	u
DQ257342.1_Morchella_esculenta	20.Oct.05	China	u
DQ257343.1_Morchella_conica	20.Oct.05	China	u
DQ257344.1_Morchella_conica	20.Oct.05	China	u
DQ257345.1_Morchella_conica	20.Oct.05	China	u
DQ257346.1_Morchella_crassipes	20.Oct.05	China	u
DQ257347.1_Morchella_crassipes	20.Oct.05	China	u
DQ491503.1_Discotis_venosa	11.Apr.06	Not given	u
EF062475.1_Morchella_elata	15.Oct.06	Not given	u
EF419956.1_Fungal_endophyte_is	01.Feb.07	USA, Arizona	u
U51851.2_Morchella_esculenta	CD 1987	Northern-Alsace, France	u
U51852.2_Morchella_conica	08.Sept.03	Graubunden, Switzerland	4

(Left) This table contains information on all of the samples from GenBank. Most are from ouside North America. "CD" before the date of collection means actually collected on that date; other dates represent the GenBank accession date when no collection date could be determined.

(Below) This table contains information on all of our samples. Note that even though we could not determine a definite geographic location for three of the samples, they were probably still from Interior Alaska. The first three samples Ian Herriott collected from the soil in a previous study.

Identification on Tree	Collection Date	Geographic Location	Habitat	Reference
EF434146.1_Uncultured_fungus	12.Feb.2007	Interior AK, Bonanza Creek LTER, site FPSC	Soil of spruce forest, not burned	(3)
IHsnow2582	May.2005	Interior AK, Estle Connector Trail Long Term site, UAF	Soil of spruce forest, not burned	unpublished
IHsnow3674	May.2005	Interior AK, Estle Connector Trail Long Term site, UAF	Soil of spruce forest, not burned	unpublished
KSR12_(GAL)17576	28.Jun.2005	Interior AK, 2004 Boundary fire	Spruce forest, burned	This study
KSR10_GAL17577	28.Jun.2005	Interior AK, 2004 Bolgen Creek fire	Spruce forest, burned	This study
KSR13_GAL17578	28.Jun.2005	Interior AK, 2004 Bolgen Creek fire	Spruce forest, burned	This study
KSR5_GAL16217	May.2004	Interior AK, Placer, off Gilmore Trail	Wild iris beds, not burned	This study
KSR11_IHM_UAF_(GAL20701)	31.May.2007	Interior AK, University of Alaska Fairbanks Campus	Edge of sidewalk, landscaping	This study
KSR1_Morel1 (GAL20707)	16.Jun.2007	Interior AK, 2006 Nenana fire	Spruce forest, burned	This study
KSR2_Morel2 (GAL20705)	16.Jun.2007	Inerior AK, 2006 Nenana fire	Spruce forest, burned	This study
KSR6_GAL20388		None given	Burn	This study
KSR7_(GAL)20410		None given	Burn	This study
KSR8_GAL20411		None given	Burn	This study
KSR9_Gyromitra	27.May.2007	Interior AK, 1524 Whistling Swan Drive, Fairbanks	Disturbed roadside	This study

METHODS

We extracted DNA from 13 dried morel samples from Dr. Gary Laursen's herbarium using the ENZA fungal kit, and copied a fragment of DNA using Polymerase Chain Reaction (PCR). We then conducted gel electrophoresis, a method used to show the amount and quality of DNA in a PCR sample. We purified the PCR products using the Zymo DNA Clean and Concentrator™-5 kit. Next, we ran a cycle sequencing reaction, in which the order of nitrogenous bases (the genetic code) is determined. We ran 50 cycles and then purified the results, and submitted the results to the UAF DNA core lab facility, where the samples were run on a 3130XL genetic analyzer. After receiving the data, we imported it to a computer and assembled it using Sequencher, then aligned it using Clustal. We also gathered additional morel samples from GenBank, an online database containing tens of millions of DNA sequences. Our final step involved building a phylogenetic tree of our samples along with those from GenBank using various algorithms (e.g. Parsimony, Neighbor Joining and Maximum Liklihood). (Background photo by Ian Herriott)

RESULTS

Our phylogenetic tree is displayed in Figure 1. There is strong support for a monophyletic (all descendents from a single common ancestor) *Morchella* genus with *Gyromytra* as our outgroup. We found that *Verpa* and *Discoti*, sister genera to *Morchella*, had only two and one named species, respectively, in GenBank. Within *Morchella*, major subdivisions exist. We have samples closely related to the major group containing what are called *M. conica*, *M. elata*, and *M. costata*, often called "Blacks." Interestingly, none of our samples are contained within the major goup known as "Yellows," and we did not have any members of *M. gigas*. Within the "black" goup, we found close relationships between DNA isolated directly from soil (IHsnow2582, IHsnow3674, EF434146.1) with DNA from sporocarps (KSR5_GAL16217, KSR6_GAL20388). KSR7_GAL20410 and KSR8_GAL2411 group with Genbank samples identified as M. conica. Samples 1, 10, 12 and 13 form a tight, distinct group. One member of the this group looks much different from the others and was identified as what has recently been called a "fuzzy foot morel," while the rest appeared to be "gray morels." However, our tree shows there is no genetic distinction, and the "fuzzy foot" is simply an immature gray morel, which supports a recent field observation (Pilz et al). We also discovered several samples from GenBank whose species attributions appear incorrect.

DISCUSSION

WHERE ARE THE ESCULENTAS AND OTHER YELLOW MORELS?

The absence of yellow morels in our samples hints that there are few, if any, in Alaska, though the geographic extent and total number of samples in this study is small. During 2005 in a burn just north of the Yukon near Canada, I picked a thousand or more morels and have never seen anything resembling *M. esculenta*, which is one of the most distinctive species groups, nor have I heard of any being obtained by any fellow morel gatherers. However, absence of evidence is not evidence of absence, and extensive further sampling is needed throughout Alaska and Canada before a "complete" picture of morel geographic diversity can be painted. This study represents a first step in that direction.

(BURNED vs. DISTURBED HABITATS, ABOVE vs. BELOWGROUND)

To our knowledge this is the first report of DNA sequences from a morel species to be found both aboveground and belowground. Detecting and identifying morel DNA from soil is important to understanding their ecology during the majority of their lifecycle. All soil sequences reported here were closely related and, interestingly, grouped with the only two aboveground morels that came from non-burned habitats (KSR5_GAL16217 – from wild iris beds near an old log pile, and KSR11_(GAL20701) from bare soil near a landscaped area on the UAF campus). However one of the 6 total Alaskan samples from this group did come from a burn (KSR5_GAL20388). Much further Alaskan sampling above and belowground, in burns and non-burns, coupled with habitat information is needed to establish confidence in any possible ecological patterns.

ORIGIN OF THE FUZZY FOOT/GRAY MORELS

The phylogenetic algorithms used to infer our phylogenies all resulted in similar trees. The Maximum Liklihood tree we show in Figure 1 is representative. However, different trees often placed the fuzzy foot group on different branches. Some placed the group near the "trunk" (as in Figure 1). Since branches closer to the base mean older in evolutionary history, this suggests that the group diverged early. However, other trees placed the group in with the "blacks" but on an unusually long branch (meaning fast evolution occured). Because small, isolated populations tend to evolve faster than other populations, one hypothesis could be that the fuzzy foot group was trapped in refugia and underwent a genetic "bottleneck", possibly during the last Ice Age. However, fuzzy foots are found in the contiguous U.S., and a comparison between these two groups is needed to test this and other hypotheses.

CONCLUSION

Some samples from GenBank have inconsistently applied "species names," shown by the fact that they are more similar genetically to members of different evolutionary groups than to the "species" to which they are attributed. This shows how little we know about morel taxonomy and the difficulty of applying morphological identifications to morels that vary widely in appearance across their lifespan. While much needs to be learned about morel biology and ecology, we first must understand what species we are discussing, or otherwise communicating study results is useless. Future studies on morel genetic diversity should involve sequencing a different gene as well as a larger and more diverse array of samples, allowing us to obtain a clearer and more concise picture of emerging morel evolution and ecology.

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