

Training 1.

How to make Google slides for ID

By Bitty Roy

Google Slides helps us:

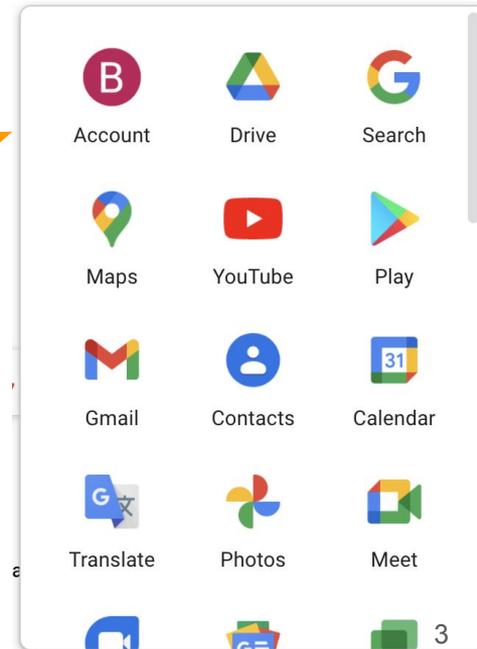
- Put all relevant information and web links in one place
- Makes it easy for different people to add or edit information
- Documents reasoning behind selecting an ID/name
- Reduces mail inbox overload and long email strings
- Can be shared with submitters who have high skill levels

To make a slideshow, first open google as your web browser; the screen will look like this

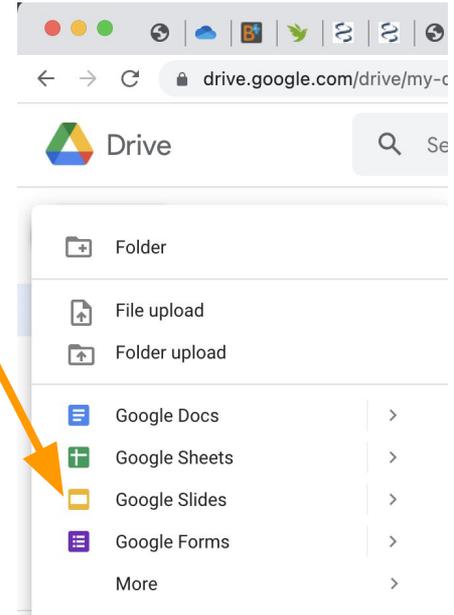
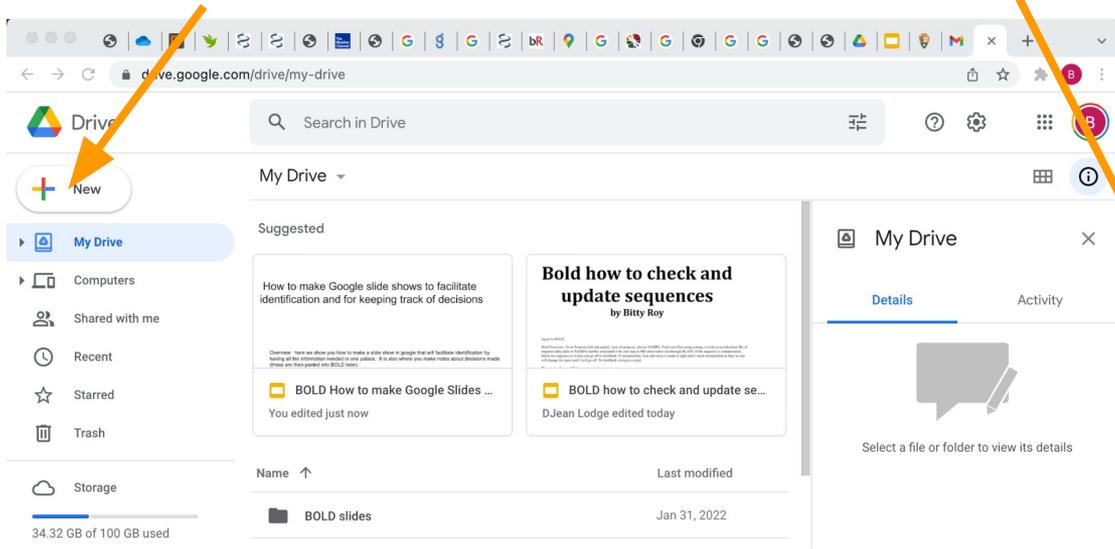


Google

1. Touch this grid of spots,
2. It will open this box, touch drive (in top line)



1. Hit +New which will open up 2. Menu choices. Select Google slides.

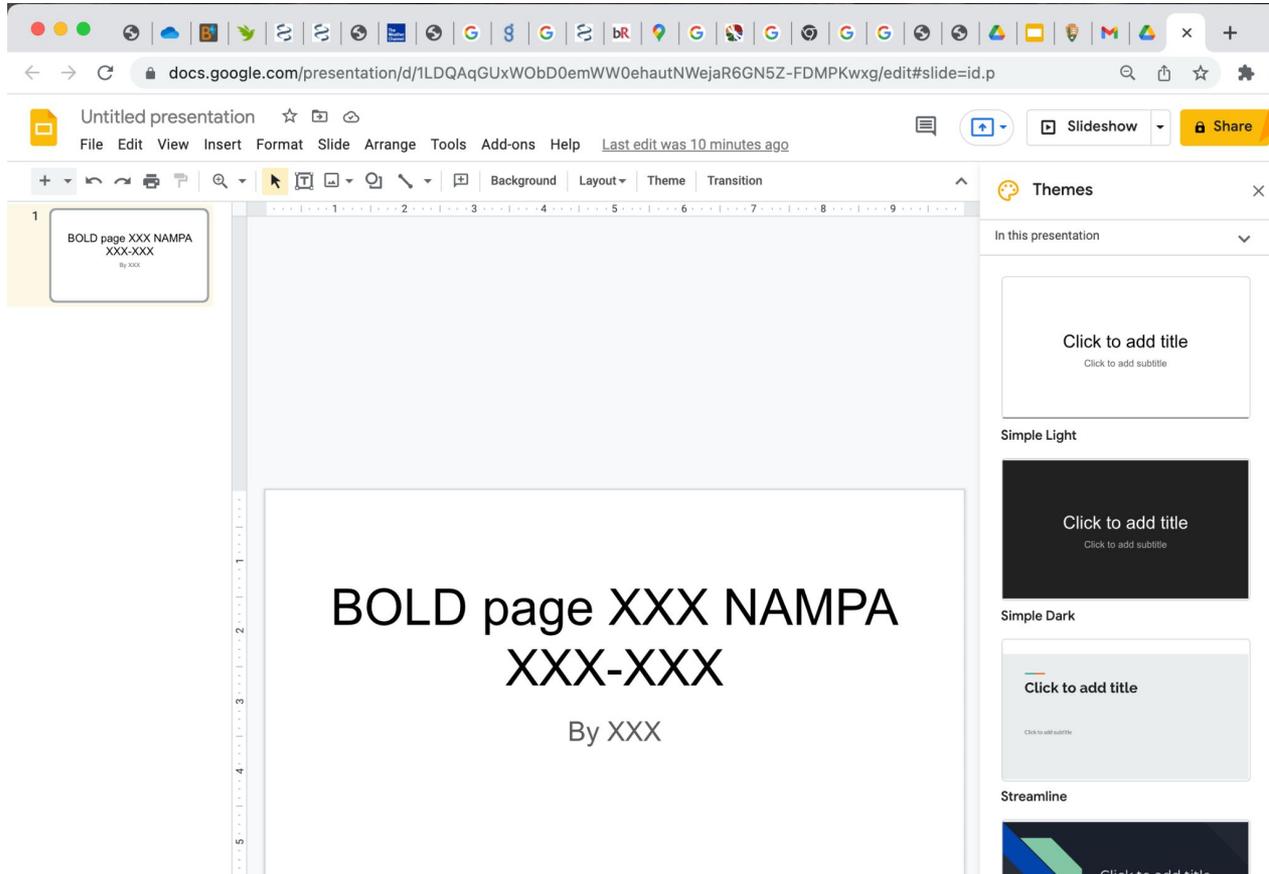


Fill in the title page with the NAMPA observations you are starting and ending with and the slide show page number (this depends on the number you are starting with). For example, if the next series that needs to be done is 1500-1599, the page number would be 16. Note that each slide show contains a maximum of 100 observations, and to keep things simple for record keeping, we start with the even hundred and end with the 99. All the sequences used to be in one big file called NAMPA, but now sometimes there are subsets taken out. When this happens, just skip the subsetted ones but DO NOT make the slideshow include other numbers than the set x-x99. Put your name under the title.

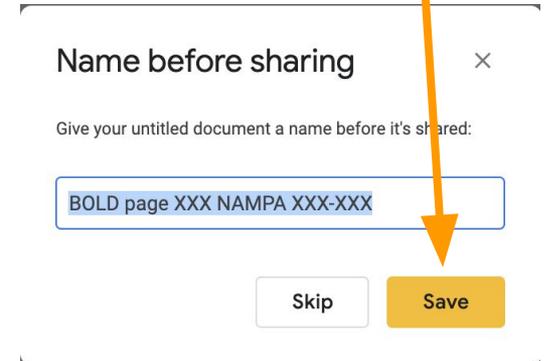
**BOLD page XXX NAMPA
XXX-XXX**

By XXX

Before you go any further, please share the slide show with the other members of the team (currently Bitty & Jean). You do this by 1. hitting the share button in the upper right.



2. This little box will pop up, asking for a title. It will automatically fill with the title you put in the slide show. Hit save



3. Next add in the email addresses of the people you wish to share it with (Google addresses work best; you'll need to answer an email giving permission if not a google address) and add a little text about why they are getting an email. 4. Make sure you toggle the function list to Editor if you want them to be able to edit the file. Hit send. Now you have a blank slide.

Share with people and groups

Jean Lodge X Bitty Roy X | Editor ▾

Notify people

Message
This is the next slideshow for identification, numbers XXX-XXX.

[Send feedback to Google](#) Cancel Send

The next step will seem odd, but it will clear the automatic formatting of google, which make making all the rest of the slides easier. See the two control boxes on this slide? You want to delete those. Touch each one to highlight it, then hit delete

Click to add title

Click to add text

Now you have a blank slide; before typing anything in it, copy and paste the blank slide 100 times (Bitty usually does this 25 times, then copies all of them and pastes all of them 3 times times to get to 100 quicker).

Inocybe cinnamomea	iNat91992095	NAMPA1500-21	1052		📍	1	2	📊				651[0n]
Ramaria rasilispora	iNat91993704	NAMPA1501-21	1052		📍	1	2	📊				696[1n]
Clavariadelphus limula	iNat91995684	NAMPA1502-21	1052		📍	1	2	📊				638[0n]

Now go to BOLD, to the first of the hundred slides you need to make, here we will choose NAMPA1500-21. Remember to sort by the NAMPA numbers, and start at the next 01 and end at 99 (follow the same pattern for larger numbers). If there is no equivalent of an 00, start with the next available number in the series, but end at the 1599 as planned. The gaps in the numbers exist because some observations have been subsetting out; ignore these holes but pretend they are part of the 100 in the slide show for counting purposes. The 2 slides show you how to set up the pairs of slides. The first slide of each observation will have the BLAST results and the second will have the distance tree associated with those results. After the example, we will show you how to make these.

This is the NAMPA number copied directly out of the BOLD Spreadsheet-a live link

This is the live iNat link. To get it copy the number out of the BOLD spreadsheet, put it into iNat and then copy the link (see later slide for how)

These are the top 10 closest "hits" from GenBank

This gap between the live links and the GenBank info is important as you will be writing notes into it

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Uncultured Inocybe note PCR # TAM2014 18S ribosomal RNA gene, partial sequence; internal transcribed sp...	uncultured Inocybe	1186	1186	98%	0.0	100.00%	642	AY310819.1
Uncultured Inocybe clone AAM_A857 18S ribosomal RNA gene, partial sequence; internal transcribed spacer ...	uncultured Inocybe	996	996	85%	0.0	98.92%	599	KF041420.1
Uncultured Inocybe clone AAM_cs:90,24 internal transcribed spacer 1, partial sequence; 5.8S ribos...	uncultured Inocybe	588	588	63%	0.0	95.26%	424	KF041378.1
Uncultured Inocybe clone 77 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA g...	uncultured Inocybe	198	198	99%	0.0	94.15%	791	KJ769273.1
NA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete s...	uncultured Inocybe	179	179	98%	0.0	94.06%	654	LC035199.1
NA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete s...	uncultured Inocybe	177	177	98%	0.0	94.05%	653	LC035200.1
P32_B24 18S ribosomal RNA gene, partial sequence; internal...	uncultured Inocybe	166	166	97%	0.0	94.00%	647	LC035201.1
18S ribosomal RNA, partial sequence; internal transcribed sp...	uncultured Inocybe	148	148	100%	0.0	93.16%	687	JX630662.1
anomic DNA containing ITS1, 5.8S rRNA gene, ITS2, clone mo...	uncultured Inocybe	154	154	89%	0.0	93.11%	599	HE820641.1

Info from the top two closest matches. These can be expanded by the id'er when needed

```

AY310819.1
642 bp DNA linear ENV 22-DEC-2010
Uncultured Inocybe note PCR # TAM2014 18S ribosomal RNA gene,
partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA
gene, and internal transcribed spacer 2, complete sequence; and 28S
ribosomal RNA gene, partial sequence.
AY310819
AY310819.1
ENV.
uncultured Inocybe
uncultured Inocybe
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina;
Agaricomycetes; Agaricomycetidae; Agaricales; Cortinariaceae;
environmental samples.
1 (bases 1 to 642)
Kennedy,P.G., Izso,A.D. and Bruns,T.D.
High potential for common mycorrhizal networks between understory
and canopy trees in a mixed evergreen forest
Unpublished
2 (bases 1 to 642)
Kennedy,P.G., Izso,A.D. and Bruns,T.D.
Direct Submission
Submitted (30-MAY-2003) Department of Plant and Microbial Biology,
University of California at Berkeley, 321 Koshland Hall, Berkeley,
CA 94720-1102, USA
Location/Qualifiers
1..642
/organism="uncultured Inocybe"
/mol_type="genomic DNA"
/isolation_source="ectomycorrhizal root tip"
/db_xref="taxon:450312"
/environmental_sample
/country="USA"
/size="cf. Inocybe sp. 13, (Ryberg et al., 2008, Mining
metadatas from unidentified ITS sequences in GenBank: A case
study in Inocybe (Basidiomycota), BMC Evolutionary Biology
8:50);
    
```

```

KF041420.1
599 bp DNA linear ENV 30-AUG-2016
Uncultured Inocybe clone AAM_A857 18S ribosomal RNA gene, partial
sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA
gene, complete sequence; and internal transcribed spacer 2, partial
sequence.
KF041420
KF041420.1
ENV.
uncultured Inocybe
uncultured Inocybe
Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina;
Agaricomycetes; Agaricomycetidae; Agaricales; Cortinariaceae;
environmental samples.
1 (bases 1 to 599)
Arguelles-Moyao,A., Garibay-Orijel,R., Marquez-Valdelamar,L.M. and
Arellano-Torres,E.
Clavulina-Membranaceus is the most important lineage within the
highly diverse ectomycorrhizal fungal community of Abies religiosa
Mycorrhiza (2016) In press
27362509
Publication Status: Available-Online prior to print
2 (bases 1 to 599)
Arguelles-Moyao,A. and Garibay-Orijel,R.
Direct Submission
Submitted (14-MAY-2013) Botany, Instituto de Biología, Universidad
Nacional Autónoma de México, Tercer Circuito s/n, Ciudad
Universitaria, D.F., D.F. 04510, México
##Assembly-Data-START##
Assembly Method : Geneious v. 5.5.6
Sequencing Technology : Sanger dideoxy sequencing
##Assembly-Data-END##
Location/Qualifiers
1..599
/organism="uncultured Inocybe"
/mol_type="genomic DNA"
/isolation_source="root tip"
/host="Abies religiosa"
/db_xref="taxon:450312"
/clone="AAM_A857"
/environmental_sample
/country="Mexico: Estado de Mexico"
    
```

This is the Distance Tree produced in GenBank. Directions on how to maximize information in the tree are further along.

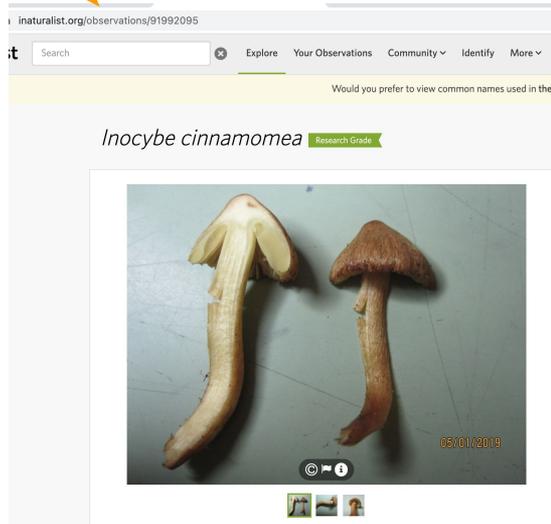
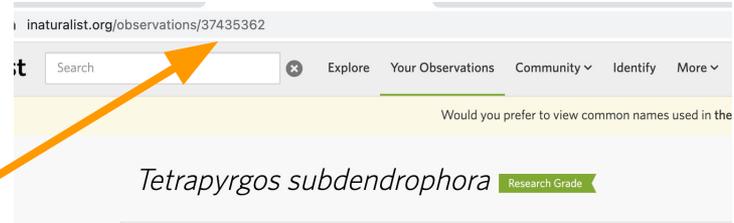


- Copy the NAMPA number right out of the BOLD spreadsheet. This will import a hotlink straight into the slide. Place this number at the top of the slide in the upper left, then hit the spacebar a few times to make a gap for the next thing you paste in. The reason why you were asked to get rid of the google formatting in the previous steps is that otherwise, these numbers are very large and they take up too much space on the slide and you have to fiddle each one to shrink it.
- Import a live link from iNaturalist (or MO—same process). To get the link, first copy just the number part following the iNat letters in BOLD, then paste this into another iNaturalist observation's numbers and hit return, then copy and paste this link (see next slide). Pasting will import the link straight into the slide. It does not work to simply change the number on a previous link, you really have to get the right link.
- Duplicate this slide that only has live links in it by going to the menu at the top of the page and selecting duplicate under the word slide
- Ok, now you have a choice, you can either finish this pair of slides before moving onto the next observation, or you can be more efficient and prepare all the slides with the NAMPA and observation numbers and then add in the BLAST and Distance trees.

Inocybe cinnamomea	iNat91992095	NAMPA1500-21	1052		1	2					651[0n]
Ramaria rasilispora	iNat91993704	NAMPA1501-21	1052		1	2					696[1n]
Clavariadelphus ligula	iNat91995684	NAMPA1502-21	1052		1	2					638[0n]

To get the live link out of iNat (or MO):

1. open the APP on your computer.
2. Open ANY observation
3. Paste the iNAt number you just copied out of BOLD (previous slide) on top of this observation's number
4. Now you will have the observation that you want the link for; copy and paste that whole link into your slide, placing it right after the BOLD NAMPA number. Hit return and the link is now live. Return to previous slide for steps 3&4.



HOW TO BLAST

BOLD SYSTEMS

Project & Dataset Search Code Record Search

Welcome to BOLD Systems

Home / BOLD Main Console

1. Go to projects

Projects: 5 Projects with access

Records: 1410 Records with access

Barcodes: 897 Barcodes with access

Sequences Traces Images

Primers Publication Checklist

Your Datasets: 1 New Dataset

Code	Title	Specimens
DS-NAMPAGB1	NAMPA Sanger Sequences for GenBank Publication Batch 1 <small>ITS[28]</small>	28

indicates a shared dataset
 indicates a shared dataset that has been publicly released
 indicates dataset ownership
 indicates dataset ownership that has been publicly released

Public Datasets

Recently Accessed Top 20

Code	Title	Specimens	Accessed
NAMPA	North American Mycoflora Project Trial Plate 2019 <small>ITS[162]</small>	475	today
DS-NAMPAGB1	NAMPA Sanger Sequences for GenBank Publication Batch 1 <small>ITS[28]</small>	28	16 days ago
GABF	Gabon Fungi <small>ITS[59]</small>	59	20+ days ago
RLCI	Reserva los Cedros Insects <small>COI-5P[620]</small>	646	20+ days ago

feedback

BOLD SYSTEMS

Project & Dataset Search Code Record Search

Activity Report

Project - NAMPA North American Mycoflora Project Trial Plate 2019

2. Choose NAMPA

Specimens

475
Specimens

GPS: 444 / 475

Country: 475 / 475

Images: 475 / 475

Barcode Compliant: 130 / 475

Sequences

162
Sequences

ITS: 162/475

Descriptors

Codes: NAMPA

Markers: ITS

Title: North American Mycoflora Project Trial Plate 2019

Description: North American Mycoflora Project (NAMP) Trial Plate 2019 through CCDB General Projects

Campaign: General Projects

Bounding Box Coordinates: N/A

Data Summary

BINS: 0

Countries (Top 5)

- United States (473)
- Canada (2)

Taxonomy

Basidiomycota (phylum): 421
Ascomycota (phylum): 54

Issues

Seqs lacking successful traces

- ITS [2]

Seqs with stop codons

- 0

User Access

Manager

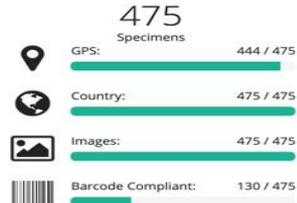
- D JEAN Lodge
dlodgester@gmail.com

Users

- Megan A. Milton
mmilton@boldsystems.or

Record List - NAMPA

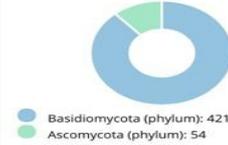
Specimens



Sequences



Taxonomy



Issues

- Seqs lacking successful trac
 - ITS [2]
- Seqs with stop codons
 - 0
- Contaminated seqs
 - 0
- Problematic records flagged
 - 0

100 records per page

Search:

Select	Identification	Specimen Page	Sequence Page	Extra Info	BIN	Record Flags	Legend	Bases [Ambig]	Tags
							ITS		
<input type="checkbox"/>	Salvatia lilacina	MO364240	NAMPA001-19			1 2	688[0n]	Mis-identified	
<input type="checkbox"/>	Amanita velosa	MO363747	NAMPA002-19			1 2	744[2n]	Validated	
<input type="checkbox"/>	Lactarius rufulus	MO364303	NAMPA003-19			1 2	715[0n]	Validated	
<input type="checkbox"/>	Fuscoporia gilva	MO363740	NAMPA004-19			1 2	713[0n]	Validated	
<input type="checkbox"/>	Annulohyphoxylon thouarsianum	MO363721	NAMPA005-19			1 2	0		
<input type="checkbox"/>	Abortiporus biennis	MO378357	NAMPA006-19			1 2	575[1n]	Contaminated	

3. Sort by sequence page

For more than one sequence,

2. select downloads

1. select the observations you want sequences from by clicking in the box

3. select sequences, see nextpage

Barcode Compliant: 833 / 1969

Ascomycota (phylum): 130

Problematic records flagged

- 0

100 records per page

Select

Select	Identification	Specimen Page	Sequence Page	Extra Info	BIN	Record Flags	Legend	Bases [Ambig]	Tags
<input checked="" type="checkbox"/>	Gymnopus foetidus								
<input checked="" type="checkbox"/>	Clitocybe subconnexa	iNat1687853						0	failed
<input checked="" type="checkbox"/>	Cyanoboletus pulverulentus	iNat17028160	NAMPA558-21	1053				718[0n]	Validated
<input checked="" type="checkbox"/>	Lactarius chelidonium	iNat17047325	NAMPA559-21	1053				731[1n]	Jean_about_200bp_are_re

Feedback

Images: 1674 / 1969

Ascomycota (phylum): 130

Problematic records

- 0

Downloads

- Sequences
- Data Spreadsheets
- Trace Files
- Sequence Analysis

Required fields are marked in red.

Marker: ITS - Internal Transcribed Spacer 1 and 2 (5)

Alignment Options: None (use submitted alignment)

Apply Filters: Sequence Length \geq 100 bp

- Exclude Contaminants
- Exclude Records with Stop Codons
- Exclude Records Flagged as Misidentifications or errors

Labels: Voucher

- Sample ID
- Sequence/Process ID
- Field ID
- Museum ID
- Collection Code
- Institution Storing

Taxonomy >

Specimen >

Collection >

- Include Marker Code
- Include BIN URI
- GenBank Accession

Apply Parameters

4. select none for alignment

5. select sample id and process id

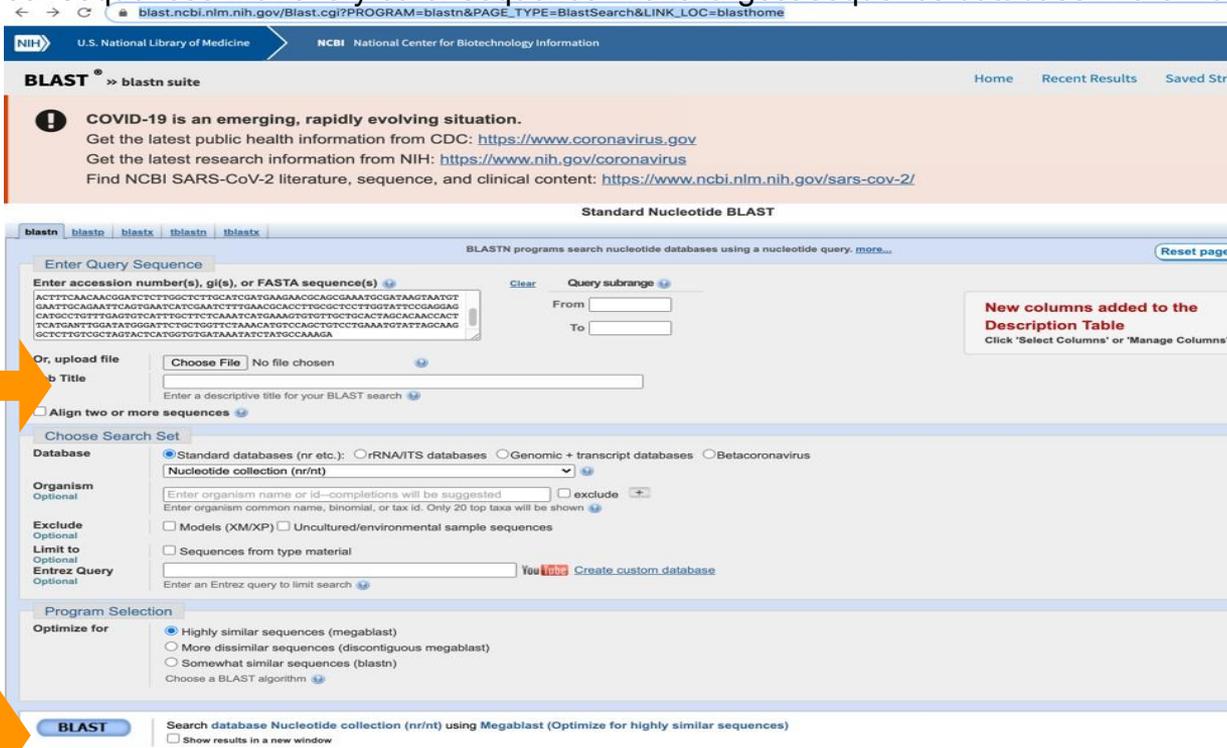
6. select apply parameters

7. Go to BLAST

This will generate a .fas (fasta) file for you and download it to your downloads. Next, Go to the GenBank and BLAST website:

https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome

In GenBank choose your fasta file to upload (8) and then hit the BLAST button (9) at the bottom of the page. Wait. This will compare your sequences with every other sequence in this largest sequence database in the world



The next section outlines how to understand the BLAST results

The list is ordered by the e-value, which is a combination of how long your bit of sequence is and the identity shared with a another. It may not be the highest percent identity. (1) For that, **sort by percent identity**. (2) Screen shot the top 10 or so sequences, including the information at the top of the table. Place this on the slide leaving a gap for notes below the id numbers. 3. Now get the information from the top two closest matches and paste those in too (see pages 18-20 for how to do this).

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
<input checked="" type="checkbox"/>	Agaricales sp. voucher DUKE:0351024 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, partial sequence	Agaricales sp.	1055	1055	100%	0.0	99.15%	719	MT138802.1
<input checked="" type="checkbox"/>	Uncultured Agaricales clone BH2117F internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, partial sequence	uncultured Agaric...	953	953	99%	0.0	96.08%	680	JF960601.1
<input checked="" type="checkbox"/>	Hygrocybe sp. voucher 110118MFBPC095 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, partial sequence	Hygrocybe sp.	952	952	100%	0.0	95.92%	721	MW554204.1
<input checked="" type="checkbox"/>	Rhodocollybia maculata voucher 130822MFBPZH0276 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, partial sequence	Rhodocollybia m...	952	952	100%	0.0	95.92%	749	MW554073.1
<input checked="" type="checkbox"/>	Rhodocollybia maculata voucher 110117MFBPL0155 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, partial sequence	Rhodocollybia m...	946	946	100%	0.0	95.75%	736	MW554383.1
<input checked="" type="checkbox"/>	Rhodocollybia maculata voucher KA13-1216 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, partial sequence	Rhodocollybia m...	944	944	99%	0.0	95.74%	709	KR673687.1
<input checked="" type="checkbox"/>	Rhodo MW862349 711 bp DNA linear PLN 11-APR-2021 er 1, partial s	Rhodocollybia maculata voucher HBAU15713 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence.	MW862349 711 bp DNA linear PLN 11-APR-2021 cribed space	4	97%	0.0	96.35%	711	MW862349.1
<input checked="" type="checkbox"/>	Rhodo MW862349 711 bp DNA linear PLN 11-APR-2021 er 1, partial s	Rhodocollybia maculata voucher HBAU15713 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence.	Rhodocollybia maculata voucher HBAU15713 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence.	5	100%	2e-159	84.71%	712	MN840644.1
<input checked="" type="checkbox"/>	Rhodo MW862349 711 bp DNA linear PLN 11-APR-2021 er 1, partial s	Rhodocollybia maculata voucher HBAU15713 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence.	Rhodocollybia maculata voucher HBAU15713 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence.	5	100%	2e-159	84.71%	708	NR_154443.1
<input checked="" type="checkbox"/>	Rhodo MW862349.1 711 bp DNA linear PLN 11-APR-2021 er 1, partial s	Rhodocollybia maculata voucher HBAU15713 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence.	Rhodocollybia maculata voucher HBAU15713 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence.	5	100%	2e-159	84.71%	708	KX834266.1

Now we are going to extract some more information out of GenBank, such as who sequenced the closest matches to ours, where those were located and if they are part of a publication. Press a sequence and a file will open up (shown on next page). Paste those shrunk onto the table of BLAST sequences.

Descriptions Graphic Summary Alignments Taxonomy

Sequences producing significant alignments Download **New** Select columns Show 100 ?

select all 100 sequences selected [GenBank](#) [Graphics](#) [Distance tree of results](#) **New** [MSA Viewer](#)

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
<input checked="" type="checkbox"/>	Agaricales sp. voucher DUKE:0351024 small subunit ribosomal RNA gene, partial sequence; internal...	Agaricales sp.	1055	1055	100%	0.0	99.15%	719	MT138802.1
<input checked="" type="checkbox"/>	Rhodocollybia maculata voucher HBAU15713 internal transcribed spacer 1, partial sequence; 5.8S ri...	Rhodocollybia...	944	944	97%	0.0	96.35%	711	MW862349.1
<input checked="" type="checkbox"/>	Uncultured Agaricales clone BH2117F internal transcribed spacer 1, partial sequence; 5.8S ribosomal...	uncultured Aga...	953	953	99%	0.0	96.08%	680	JF960601.1
<input checked="" type="checkbox"/>	Hygrocybe sp. voucher 110118MFBPC095 internal transcribed spacer 1, partial sequence; 5.8S ribos...	Hygrocybe sp.	952	952	100%	0.0	95.92%	721	MW554204.1
<input checked="" type="checkbox"/>	Rhodocollybia maculata voucher 130822MFBPZH0276 small subunit ribosomal RNA gene, partial se...	Rhodocollybia...	952	952	100%	0.0	95.92%	749	MW554073.1
<input checked="" type="checkbox"/>	Rhodocollybia maculata voucher 110117MFBPL0155 internal transcribed spacer 1, partial sequence;...	Rhodocollybia...	946	946	100%	0.0	95.75%	736	MW554383.1
<input checked="" type="checkbox"/>	Rhodocollybia maculata voucher KA13-1216 18S ribosomal RNA gene, partial sequence; internal tra...	Rhodocollybia...	944	944	99%	0.0	95.74%	709	KR673687.1
<input checked="" type="checkbox"/>	Uncultured Sclerotium genomic DNA sequence contains 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS...	uncultured Scl...	368	456	58%	4e-97	93.93%	697	LT594984.1
<input checked="" type="checkbox"/>	Entoloma sp. isolate CM13_219 small subunit ribosomal RNA gene, partial sequence; internal transcr...	Entoloma sp.	350	350	44%	1e-91	91.54%	1012	KY774199.1
<input checked="" type="checkbox"/>	Hemistropharia albocrenulata voucher S.D. Russell MycoMap # 10424 small subunit ribosomal RNA...	Hemistropharia...	344	344	43%	6e-90	91.41%	709	MN906242.1
<input checked="" type="checkbox"/>	Hemistropharia albocrenulata voucher S.D. Russell iNaturalist # 8539263 internal transcribed spacer...	Hemistropharia...	344	344	43%	6e-90	91.41%	648	MN906241.1
<input checked="" type="checkbox"/>	Hemistropharia albocrenulata voucher T15 internal transcribed spacer 1, partial sequence; 5.8S ribos...	Hemistropharia...	344	344	43%	6e-90	91.41%	611	MH697851.1
<input checked="" type="checkbox"/>	Richoniella sp. MEL 2135811 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA ge...	Richoniella sp...	346	346	44%	2e-90	91.15%	606	KP191916.1
<input checked="" type="checkbox"/>	Entoloma albidum strain YL620 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA...	Entoloma albid...	340	340	43%	8e-89	90.66%	965	KC710102.1
<input checked="" type="checkbox"/>	Myochromella boudieri voucher MushroomObserver.org/307728 internal transcribed spacer 1, partial...	Myochromella...	344	344	46%	6e-90	90.33%	928	MG890331.1
<input checked="" type="checkbox"/>	Rhodocybe brunneaurantiaca voucher CUH AM720 internal transcribed spacer 1, partial sequence;...	Rhodocybe bru...	435	435	75%	4e-117	85.26%	504	MW023201.1
<input checked="" type="checkbox"/>	Rhodocybe sp. YS-2020a voucher HMJAU56921_1 internal transcribed spacer 1, partial sequence; 5...	Rhodocybe sp...	569	569	97%	1e-157	84.92%	701	MW298803.1
<input checked="" type="checkbox"/>	Rhodocybe sp. YS-2020a voucher HMJAU56921_3 internal transcribed spacer 1, partial sequence; 5...	Rhodocybe sp...	571	571	98%	3e-158	84.88%	705	MW298805.1
<input checked="" type="checkbox"/>	Rhodocybe sp. YS-2020a voucher HMJAU56921_2 internal transcribed spacer 1, partial sequence; 5...	Rhodocybe sp...	569	569	98%	1e-157	84.85%	705	MW298804.1
<input checked="" type="checkbox"/>	Rhodocybe asyae voucher Personal collection:NA131019 internal transcribed spacer 1, partial sequ...	Rhodocybe asy...	575	575	100%	2e-159	84.71%	712	MN840644.1
<input checked="" type="checkbox"/>	Rhodocybe asyae KATO Fungi 3640 ITS region; from TYPE material	Rhodocybe asy...	575	575	100%	2e-159	84.71%	7	

Feedback

MT138802 719 bp DNA linear PLN 30-APR-2021

Agaricales sp. voucher DUKE:0351024 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence.

MT138802

MT138802.1

.

Agaricales sp.

Agaricales sp.

Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina; Agaricomycetes; Agaricomycetidae; Agaricales.

1 (bases 1 to 719)

Eberhardt,U., Schuetz,N. and Beker,H.J.

Duke collections

Unpublished

2 (bases 1 to 719)

Eberhardt,U.

Direct Submission

Submitted (04-MAR-2020) Botanik, State Museum of Natural History Stuttgart, Rosenstein 1, Stuttgart 70191, Germany

##Assembly-Data-START##

Sequencing Technology :: Sanger dideoxy sequencing

##Assembly-Data-END##

Location/Qualifiers

1..719

/organism="Agaricales sp."

/mol_type="genomic DNA"

/specimen_voucher="DUKE:0351024"

/db_xref="taxon:1756126"

/country="USA"

This box from the first sequence tells us who sent in the sequences Eberhardt et al), when (30-Apr-21), and that it is a DUKE (South Carolina, USA) collection not from Germany, though it was submitted from Germany. It does not appear to be associated with a publication (I searched "Duke Collections" fungi), in this case, which is too bad. Note this sequence was submitted in 2021. This sequence is similar to ours by 99.15%--pretty good.

MW862349 711 bp DNA linear PLN 11-APR-2021
Rhodocollybia maculata voucher HBAU15713 internal transcribed
spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal
transcribed spacer 2, complete sequence; and large subunit
ribosomal RNA gene, partial sequence.
MW862349
MW862349.1

•
Rhodocollybia maculata
[Rhodocollybia maculata](#)

Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina;
Agaricomycetes; Agaricomycetidae; Agaricales; Omphalotaceae;
Rhodocollybia.

1 (bases 1 to 711)

Shi,L.

Direct Submission

Submitted (03-APR-2021) Key Laboratory of Vegetable Germplasm
Innovation and Utilization of Hebei, College of Horticulture, Hebei
Agricultural University, lekainandajie, Baoding 071000, China

##Assembly-Data-START##

Sequencing Technology :: Sanger dideoxy sequencing

##Assembly-Data-END##

Location/Qualifiers

1..711

/organism="Rhodocollybia maculata"

/mol_type="genomic DNA"

/specimen_voucher="HBAU15713"

/db_xref="taxon:[2074862](#)"

<1..>711

/note="contains internal transcribed spacer 1, 5.8S
ribosomal RNA, internal transcribed spacer 2, and large

The second sequence was from China, but is not very close to ours, certainly not at the species level, 96.35%

Next I would press on the Distance tree of results (2) Use the fast evolution, not neighbor joining model

COVID-19 Information
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RID ZS58BCZA016 Search expires on 02-05 00:46 am [Download All](#) [Citation](#)
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Database nt [See details](#)
Query ID Icl|Query_31611
Description None
Molecule type dna
Query Length 583
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Organism only top 20 will appear exclude
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[+ Add organism](#)

Percent Identity to **E value** to **Query Coverage** to

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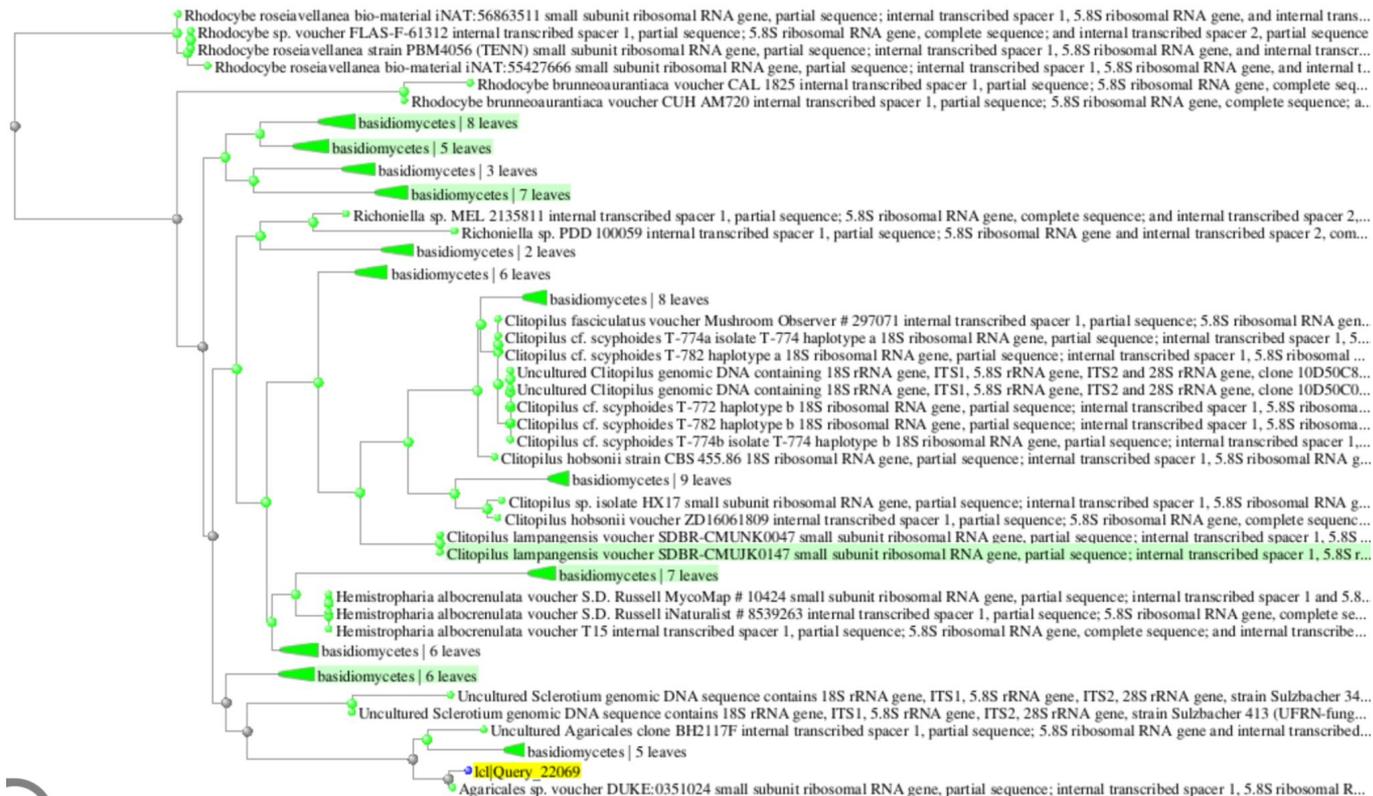
Descriptions [Graphic Summary](#) **2**

Sequences producing significant alignments [Download](#) [New](#) [Select columns](#) [Show](#) 100 [?](#)

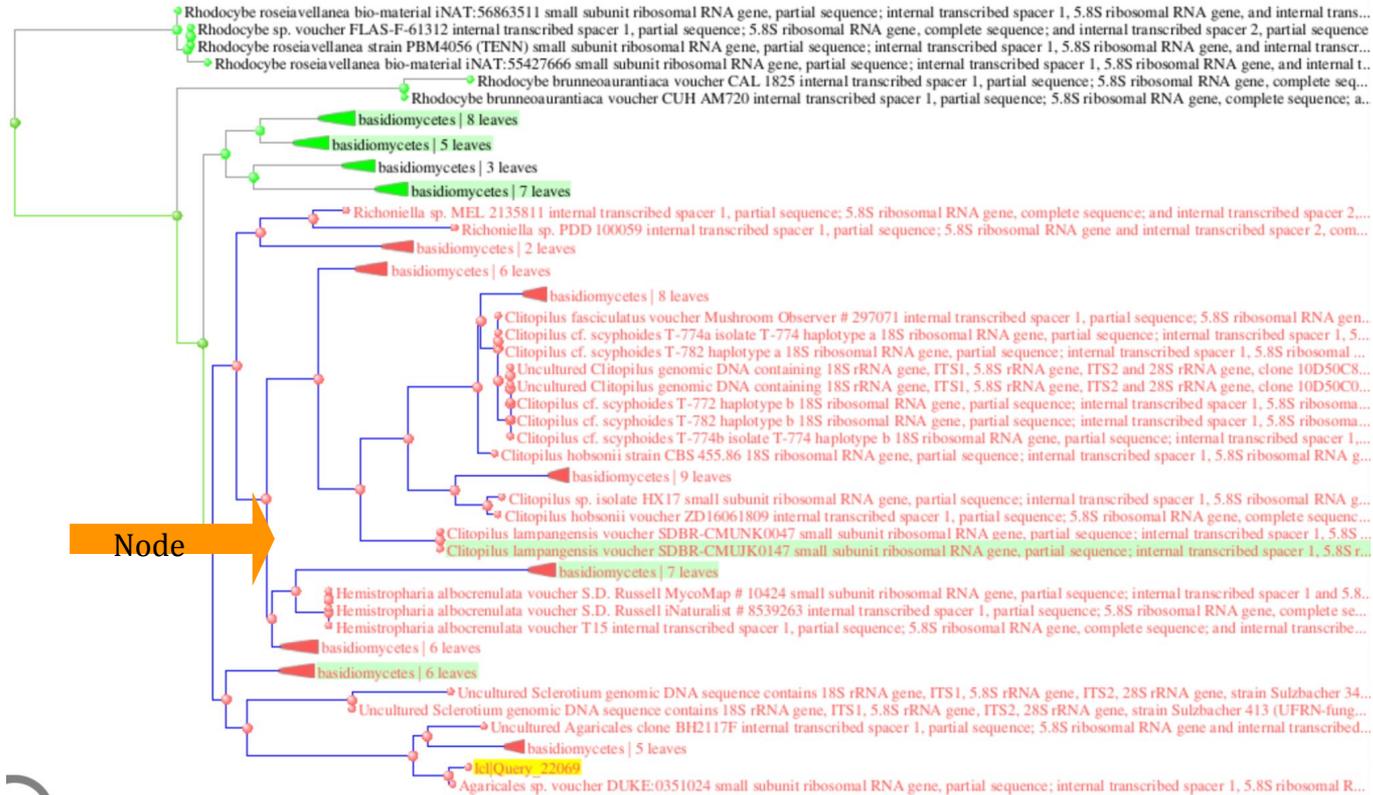
select all 100 sequences selected [GenBank](#) [Graphics](#) [Distance tree of results](#) [New](#) [MSA Viewer](#)

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
<input checked="" type="checkbox"/>	Agaricales sp. voucher DUKE:0351024 small subunit ribosomal RNA gene, partial sequence: internal transcribed s...	Agaricales sp.	1055	1055	100%	0.0	99.15%	719	MT138802.1
<input checked="" type="checkbox"/>	Uncultured Agaricales clone BH2117F internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA gene a...	uncultured Agaric...	953	953	99%	0.0	96.08%	680	JF960601.1
<input checked="" type="checkbox"/>	Hydrocybe sp. voucher 110118MFBPC095 internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA ge...	Hydrocybe sp.	952	952	100%	0.0	95.92%	721	MW554204.1
<input checked="" type="checkbox"/>	Rhodocollybia maculata voucher 130822MFBPZH0276 small subunit ribosomal RNA gene, partial sequence: inter...	Rhodocollybia m...	952	952	100%	0.0	95.92%	749	MW554073.1
<input checked="" type="checkbox"/>	Rhodocollybia maculata voucher 110117MFBPL0155 internal transcribed spacer 1, partial sequence: 5.8S ribosom...	Rhodocollybia m...	946	946	100%	0.0	95.75%	736	MW554383.1
<input checked="" type="checkbox"/>	Rhodocollybia maculata voucher KA13-1216 18S ribosomal RNA gene, partial sequence: internal transcribed spac...	Rhodocollybia m...	944	944	99%	0.0	95.74%	709	KR673687.1
<input checked="" type="checkbox"/>	Rhodocollybia maculata voucher HBAU15713 internal transcribed spacer 1, partial sequence: 5.8S ribosomal RNA...	Rhodocollybia m...	944	944	97%	0.0	96.35%	711	MW862349.1
<input checked="" type="checkbox"/>	Rhodocybe asyae voucher Personal collection:NA131019 internal transcribed spacer 1, partial sequence: 5.8S rib...	Rhodocybe asyae	575	575	100%	2e-159	84.71%	712	MN840644.1
<input checked="" type="checkbox"/>	Rhodocybe asyae KATO_Fungi_3640 ITS region: from TYPE material	Rhodocybe asyae	575	575	100%	2e-159	84.71%	708	NR_154443.3

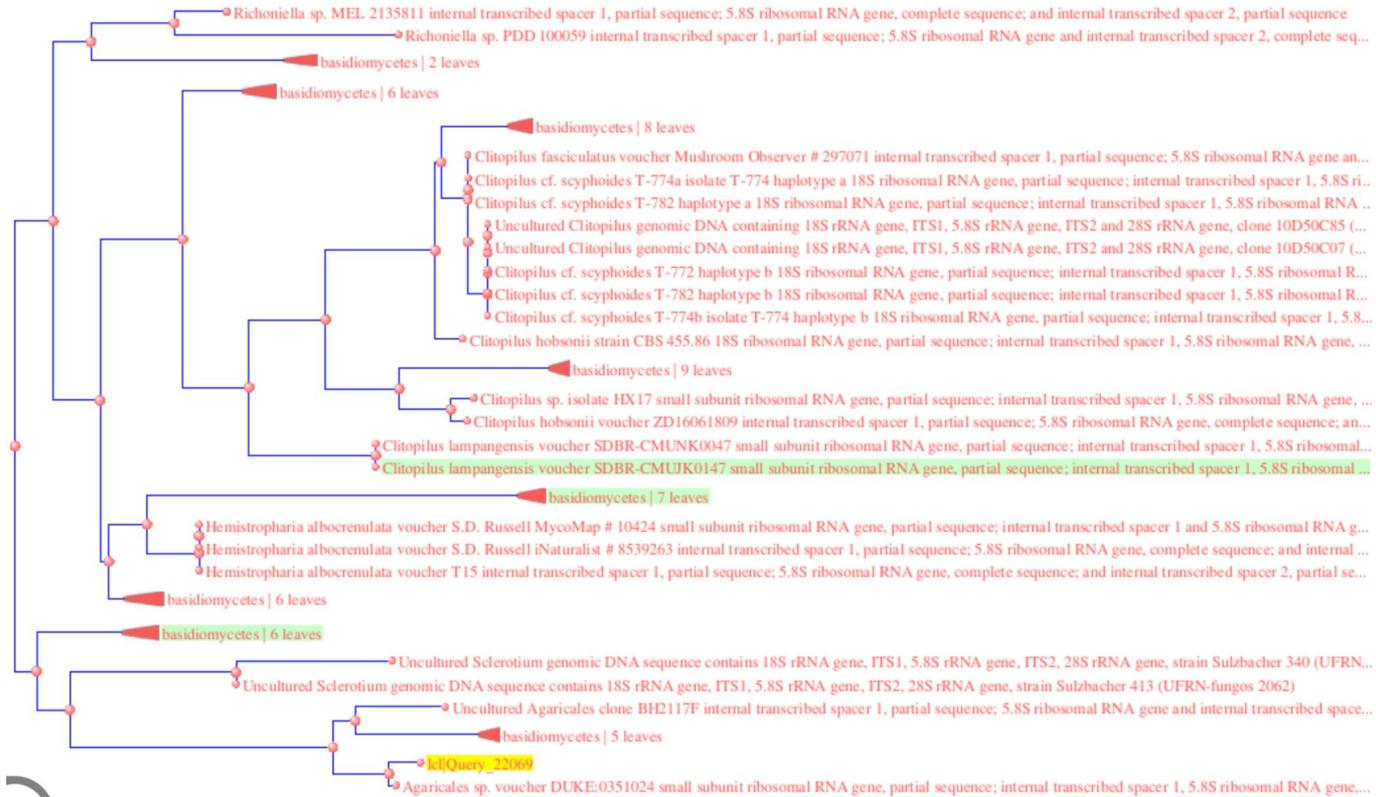
Here is the first distance tree. Unfortunately, there are a lot of places where BLAST automatically decided to subsume information (a triangle then text that says basidiomycetes x (nr) of leaves). We are going to make it give us the information, especially around our query (which is in yellow). next slide.

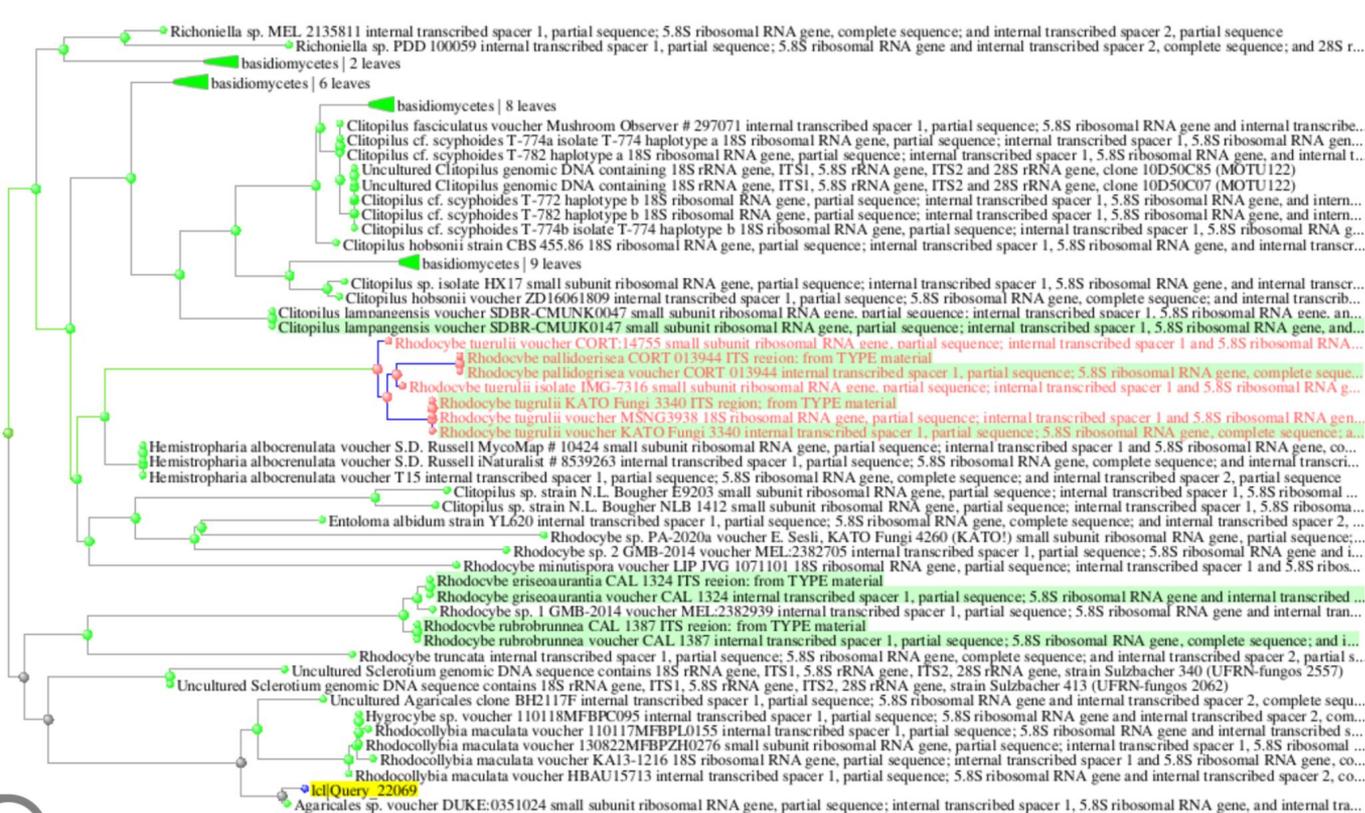


First, let's isolate just the most informative part of the tree, the part that is closest to your sample. To do that first touch the dot (called a node) with the pointer for the group of sequences next to yours. When you touch it, the node turns red, as you can see here and gives you a menu. Press show subtree (next slide)



There is still a lot of hidden information. Now to expand the individual red triangles near your query (highlighted in yellow), open up the triangles by touching them (sometimes this takes a few tries; be persistent), you will eventually get a menu of options, press expand. Next slide shows all the close by triangles opened up.





This is the image to screenshot and paste into the second slide for this observation.

An Example where the literature made a difference!

NAMPA1255-21

<https://www.inaturalist.org/observations/47210859>

NAMPA1255-21 Basidiomycota Nat 47210859	present_in	SH1830889.08FU	k_Fungi;p__Basidiomycota;c__Agaricomycetes;o__Hymenochaetales;f__Hymenochaetaceae;g__Phellinus;s__Phellinus_sp
--	------------	----------------	--

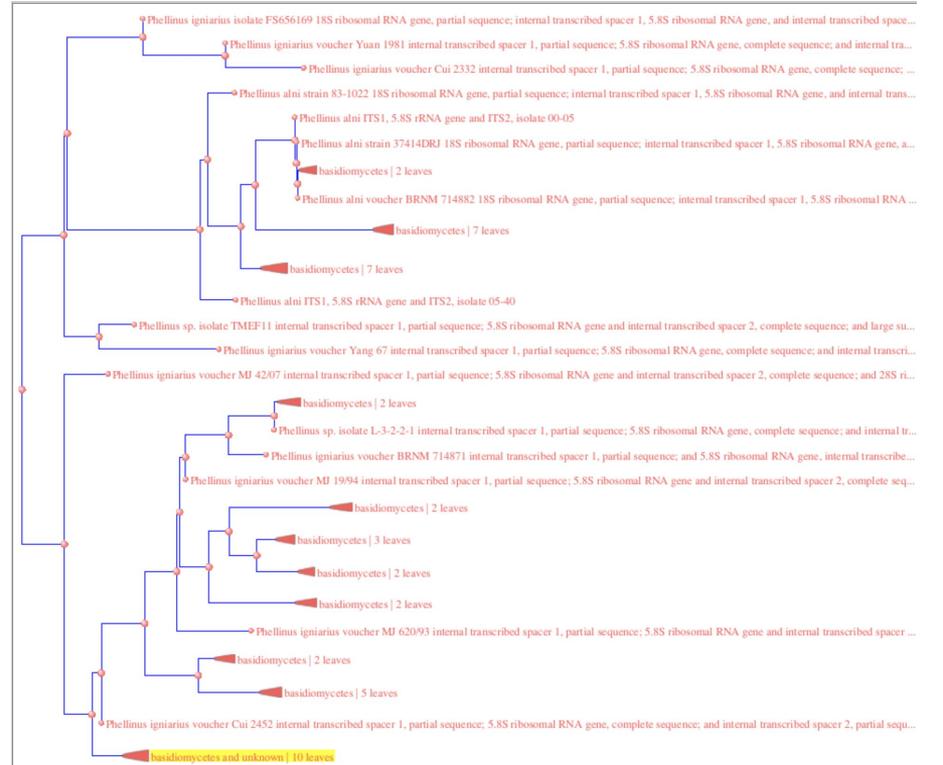
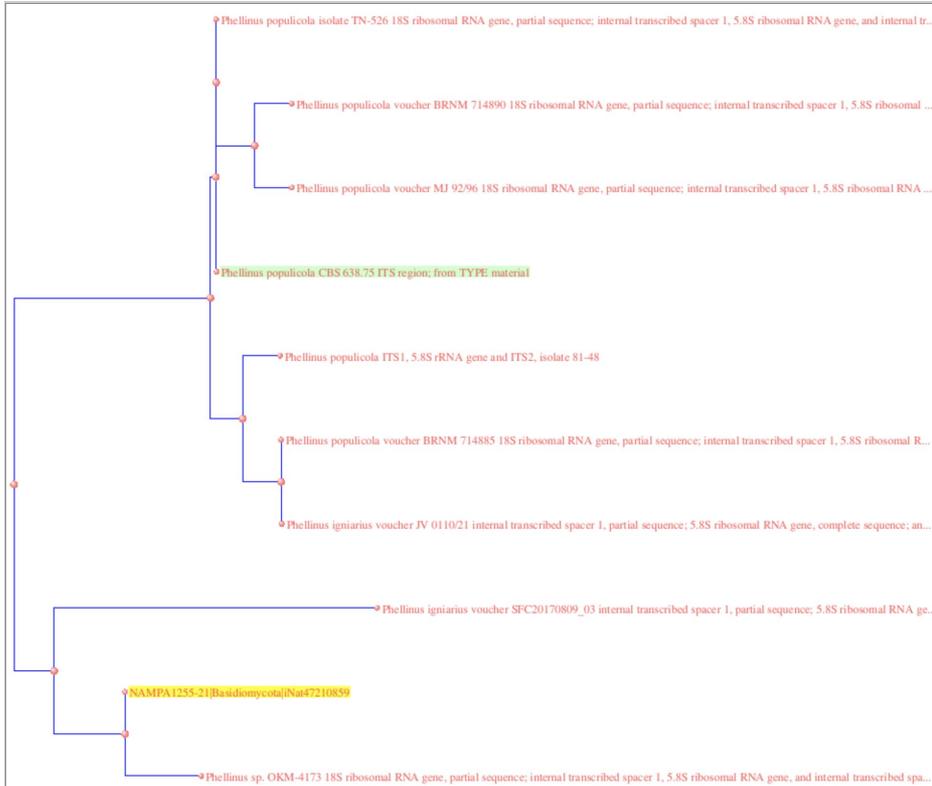
It is clear that what most people do is see that they have a hit to *P. ignarius* and assign that name to their sequence. However, if you use phylogenies you can see that there is a lot of variation in *Phellinus* and that it clusters into several species. According to Zhou et al. 2016, “Gilbertson and Ryvarden (1987) recorded *P. ignarius* in North American mycota; however, according to our field surveys and GenBank searches, there is no indication of this species in North America” pg. 200 of Zhou et al. 2016. This particular sequence is 99.78% match and 100% cover to a sequence (OKM-4173 GenBank KU139180) discussed by Brazee (2016). In Brazee’s phylogeny, it formed its own clade and lives on *Populus*. He calls it *Phellinus* NA1, according to him, “A single isolate collected from Idaho in 1966 by Orson Miller, and presumed to be *P. tremulae*, represents a sister species to *P. ignarius* s.s. and *P. populicola*. This species, *P. NA1*, remains undescribed here pending further study on its distribution and host range.” pg. 420 of Brazee 2016. This sequence and specimen should be sent to Brazee so he can describe the species!

Braze, N. J. 2015. Phylogenetic relationships among species of *Phellinus* sensu stricto, cause of white trunk rot of hardwoods, from northern North America. *Forests* 6:4191-4211.

Zhou, L.-W., J. Vlasák, W.-M. Qin, and Y.-C. Dai. 2016. Global diversity and phylogeny of the *Phellinus ignarius* complex (Hymenochaetales, Basidiomycota) with the description of five new species. *Mycologia* 108:192-204.

	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Phellinus sp. OKM-4173 18S ribosomal RNA gene, partial sequence; internal transcribed s...	Phellinus sp...	819	819	100%	0.0	99.78%	669	KU139180.1
Phellinus ignarius voucher JV 0709/142 internal transcribed spacer 1, partial sequence; 5...	Phellinus igni...	771	771	95%	0.0	99.07%	596	JX069820.1
Phellinus ignarius voucher Cui 2452 internal transcribed spacer 1, partial sequence; 5.8S r...	Phellinus igni...	761	761	95%	0.0	98.83%	596	JQ828876.1
Phellinus ignarius strain CCBAS575 internal transcribed spacer 1, partial sequence; 5.8S r...	Phellinus igni...	688	688	86%	0.0	98.71%	388	KX239476.1
Phellinus ignarius voucher BRNM 714866 18S ribosomal RNA gene, partial sequence; inte...	Phellinus igni...	750	750	97%	0.0	97.73%	627	GQ383710.1
Phellinus ignarius voucher MJ 39/07 internal transcribed spacer 1, partial sequence; 5.8S r...	Phellinus igni...	710	710	92%	0.0	97.38%	607	GQ383711.1
Phellinus ignarius voucher MJ 40/07 internal transcribed spacer 1, partial sequence; 5.8S r...	Phellinus igni...	704	704	92%	0.0	97.14%	607	GQ383712.1
Phellinus ignarius voucher MJ 42/07 internal transcribed spacer 1, partial sequence; 5.8S r...	Phellinus igni...	712	712	93%	0.0	96.97%	615	GQ383717.1
Phellinus ignarius voucher MJ 19/94 internal transcribed spacer 1, partial sequence; 5.8S r...	Phellinus igni...	702	702	92%	0.0	96.93%	610	GQ383718.1
Phellinus ignarius var. trivialis strain CBS 512.63 internal transcribed spacer 1, 5.8S riboso...	Phellinus igni...	723	723	95%	0.0	96.80%	669	AY558624.1

NAMPA1255-21 Basidiomycota Nat 47210859	present_in	SH1830889.08FU	k_Fungi;p__Basidiomycota;c__Agaricomycetes;o__Hymenochaetales;f__Hymenochaetaceae;g__Phellinus;s__Phellinus_sp
--	------------	----------------	--



NAMPA1255-21 Basidiomycota Nat	present_in	SH1830889.08FU	k_Fungi;p_Basidiomycota;c_Agaricomycetes;o_Hymenochaetales;f_Hymenochaetaceae;g_Phellinus;s_Phellinus_sp
47210859			

KU139180 669 bp DNA linear PLN 30-APR-2016
 Phellinus sp. OKM-4173 18S ribosomal RNA gene, partial sequence;
 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and
 internal transcribed spacer 2, complete sequence; and 28S ribosomal
 RNA gene, partial sequence.

KU139180
 KU139180.1

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Phellinus sp. OKM-4173
[Phellinus sp. OKM-4173](#)

Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina;
 Agaricomycetes; Hymenochaetales; Hymenochaetaceae; Phellinus;
 unclassified Phellinus.

1 (bases 1 to 669)
 Brazeo, N.J.

Phylogenetic Relationships among Species of Phellinus sensu
 stricto, Cause of White Trunk Rot of Hardwoods, from Northern North
 America

Forests 6 (11), 4191-4211 (2015)

2 (bases 1 to 669)
 Brazeo, N.J.

Direct Submission

Submitted (08-NOV-2015) Center for Agriculture, Food and the
 Environment, University of Massachusetts, 101 University Drive,
 Amherst, MA 01002, USA