**Insect DNA Barcoding Mini-project**

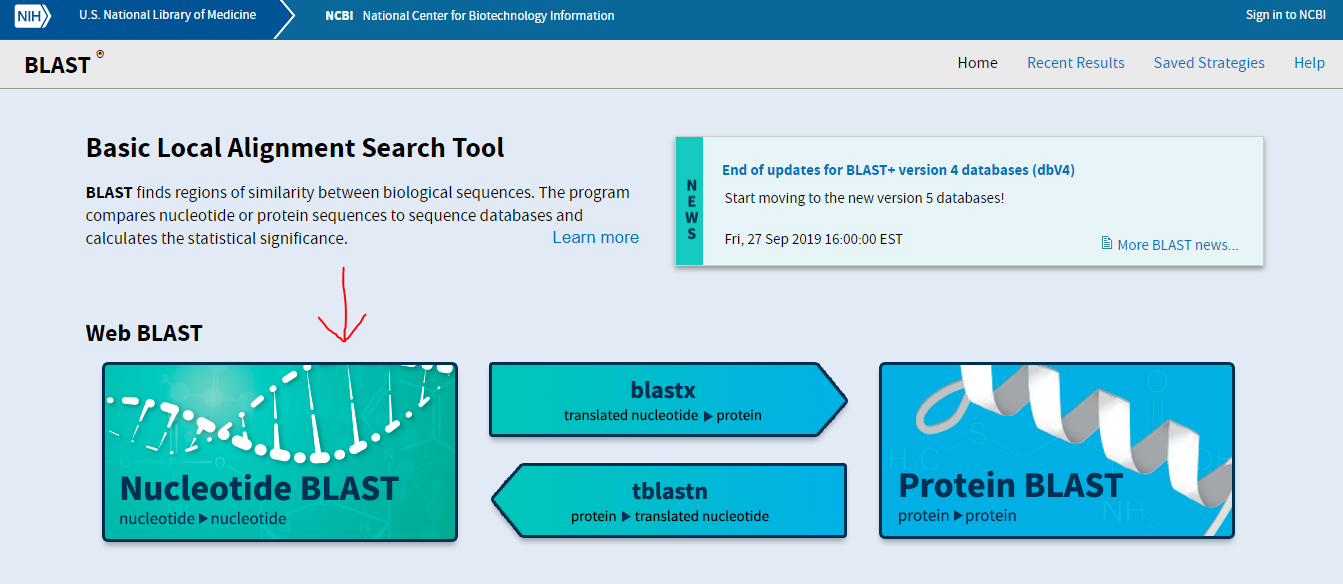
By the end of this activity, you will be able to:

* Use two sequence databases (BLAST & BOLD) to identify your unknown insect sequence collected by you or a classmate at the L.A. River
* Use the GBIF database to obtain more information on your insect
* Submit your identified insect sequence into the BOLD database through the student portal

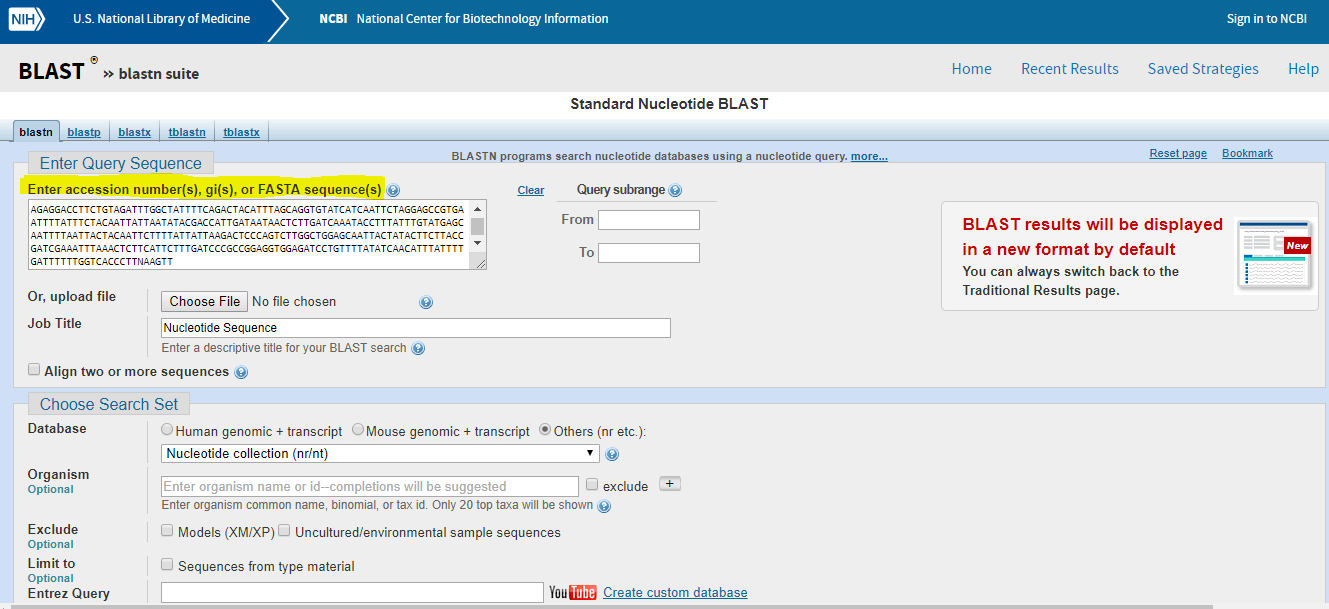
You have been assigned a sequence and you will use this worksheet to submit your answers:

(<https://docs.google.com/spreadsheets/d/1lJzQRG0GNdOTSCkGwUCSsXW2nKszPoJrjsQK5Bi9gog/edit?usp=sharing>)

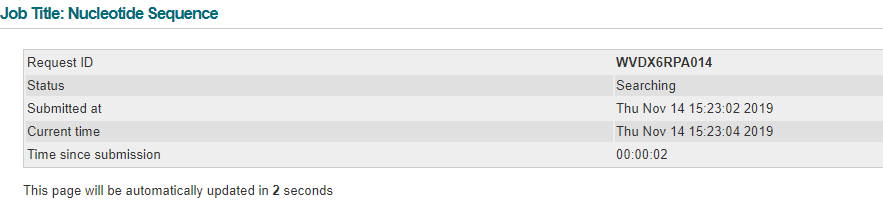
Navigate to the BLAST website (<https://blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_320521487>). Click “Nucleotide BLAST” as pictured below.



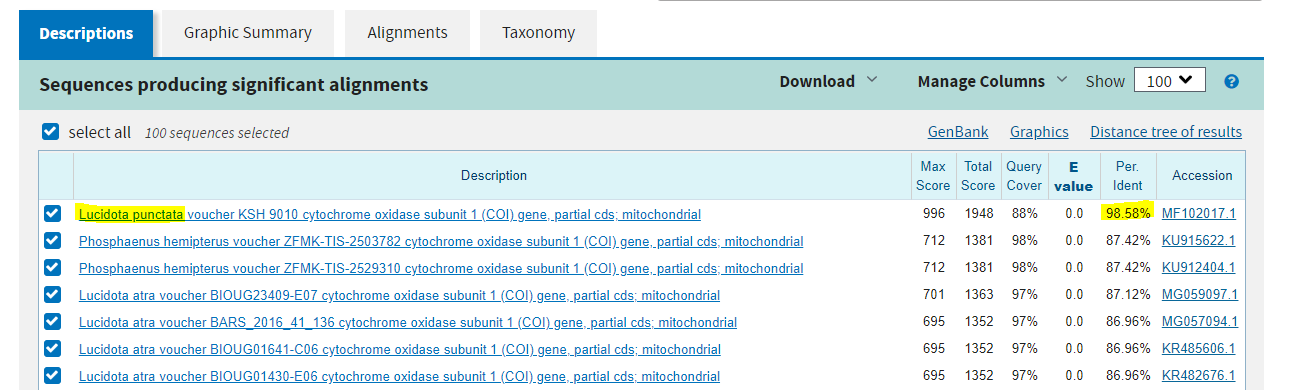
Next, where it says enter accession number(s), insert your DNA sequence.



Next, simply scroll down and click blast!

You will see the function running below, and it will take a few moments to load.

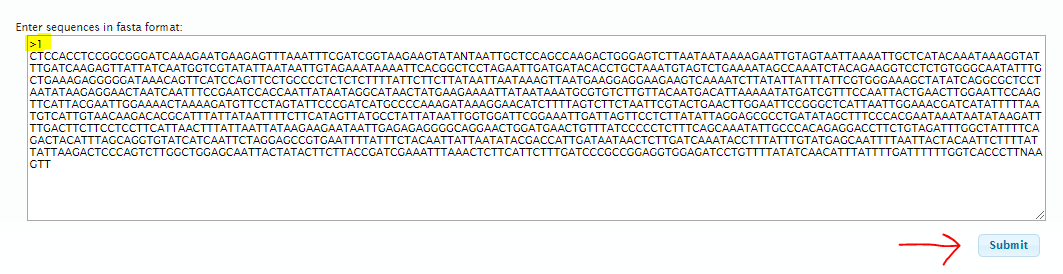
After the Blast loads, scroll down to view significant alignments.



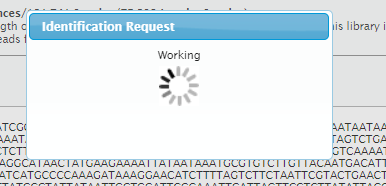
A Percent Identification of 99% or higher is preferable. **Record the suggested identification only if it has a percentage of 85 or above and add the Accession number.**

When you are finished exploring and identifying your sequence, scroll up to “Edit Search” to restart if needed.

Navigate to the BOLD website (<http://v3.boldsystems.org/index.php/IDS_OpenIdEngine>). Simply copy and paste your sequence where it says “Enter sequences in fasta format.” All this means is to run, the program needs a greater than (>) and a number (e.g. 1) ahead of the sequence. This is simply a formatting preference. See the figure below for an example.

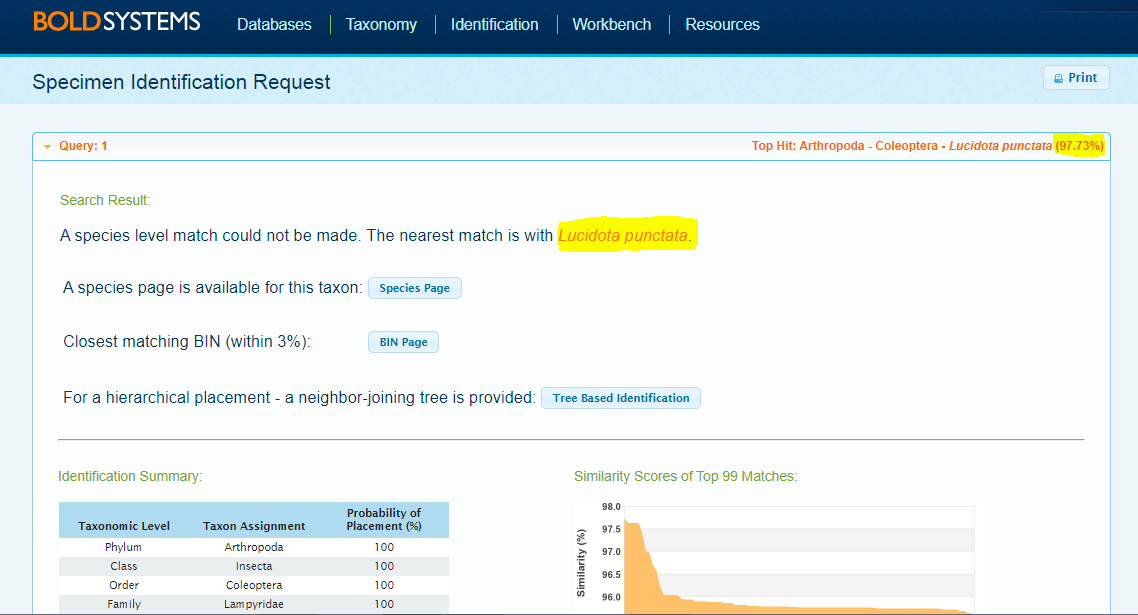


For simplicity, enter >1 followed by your sequence. Then, press submit to run.



Once BOLD searches its database, it will redirect you to a results page.

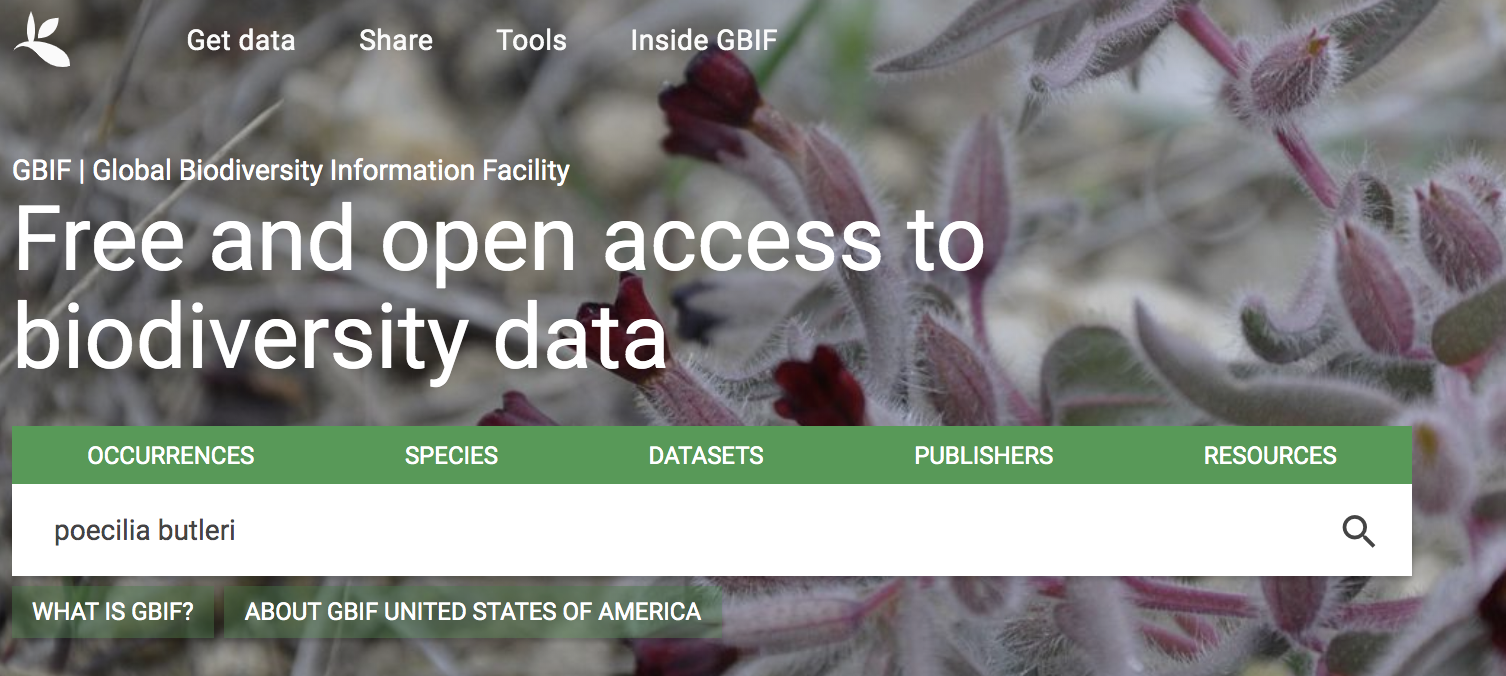
The results page will tell you if a species level match was made, the nearest match, or if no match was made at all. In the top right, the percent identification will be displayed. **Again, record the suggested identification only if it has a percentage of 85 or above.**



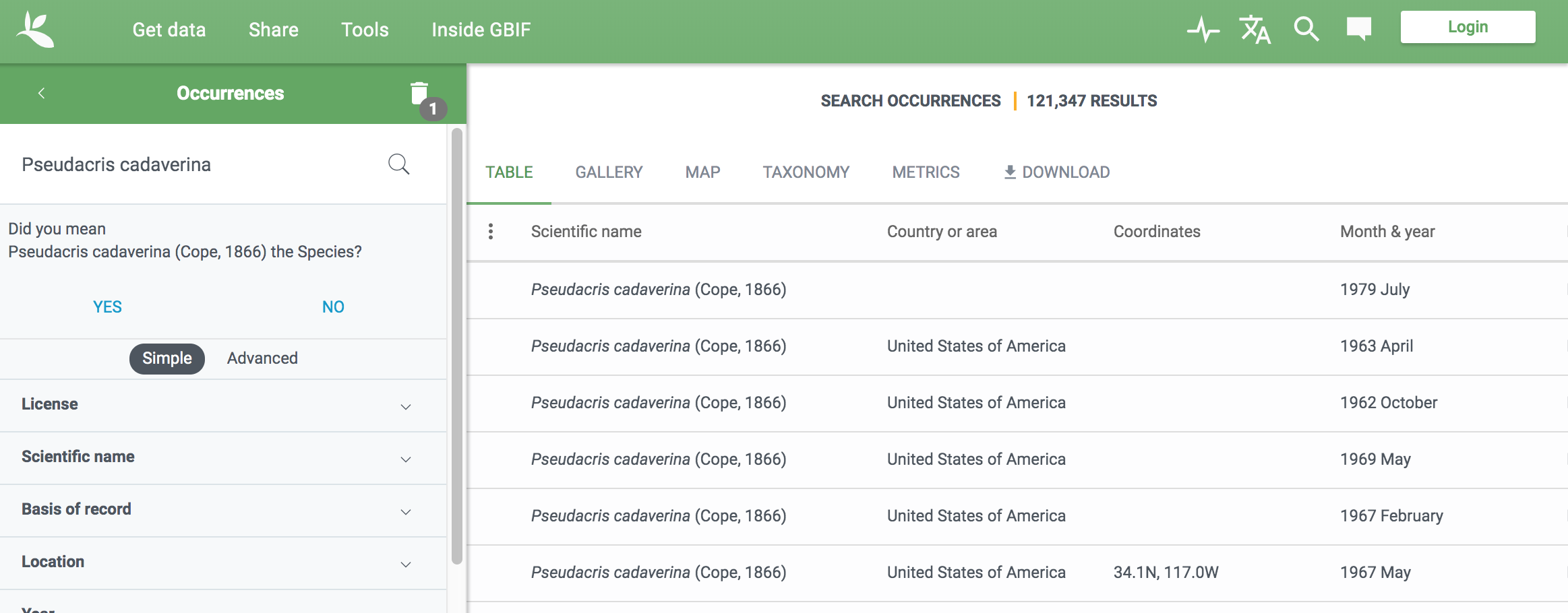
You should now have identified your organism to species or genus level! A tentative match for those who are 90% or below.

If needed, simply press the back arrow on your browser to restart.

Now go to the GBIF database <https://www.gbif.org/> and type the name of your organism into the search box and click **Occurrences**



This will take you to a page with different tabs, explore each one and record information on the Second tab of Google spreadsheet.

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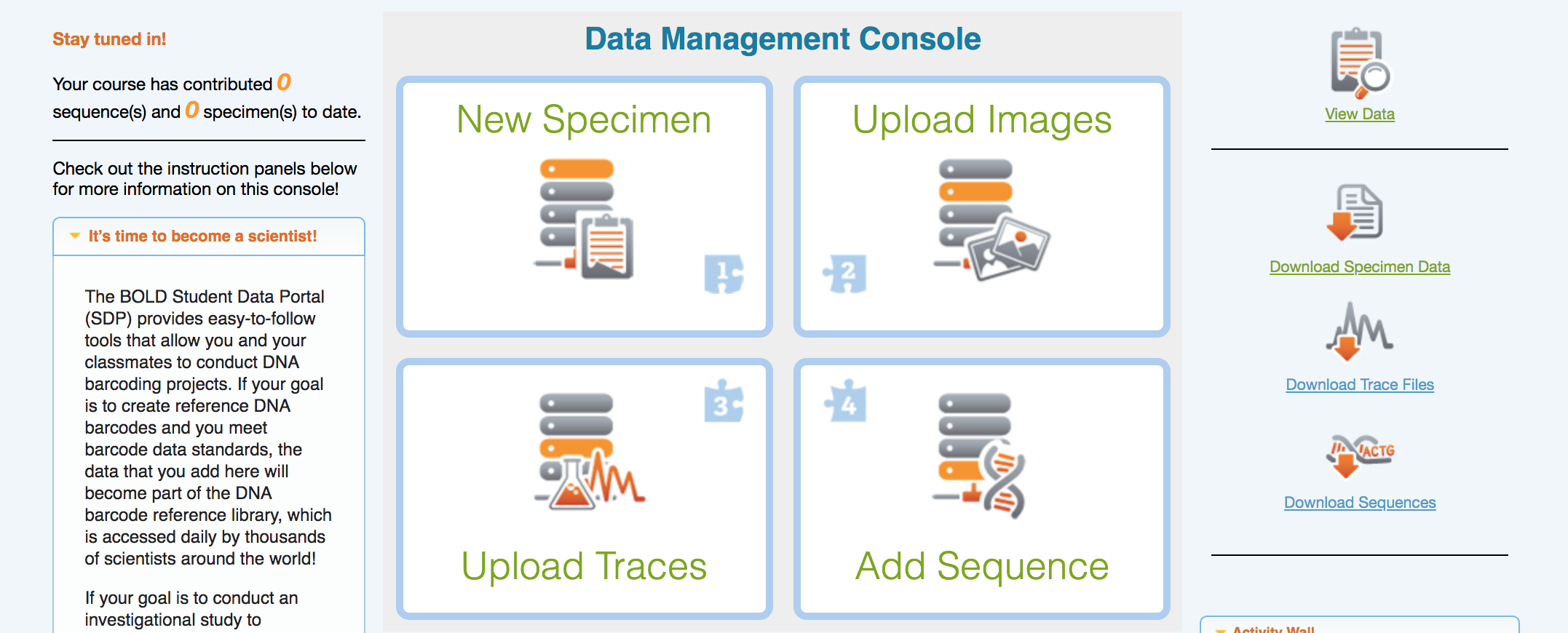
**Be sure to record specific information on the spreadsheet tab labelled GBIF!**

**BOLD Student Portal**

Now go to the BOLD student portal <http://v3.boldsystems.org/index.php/SDP_Home> and **click Students**

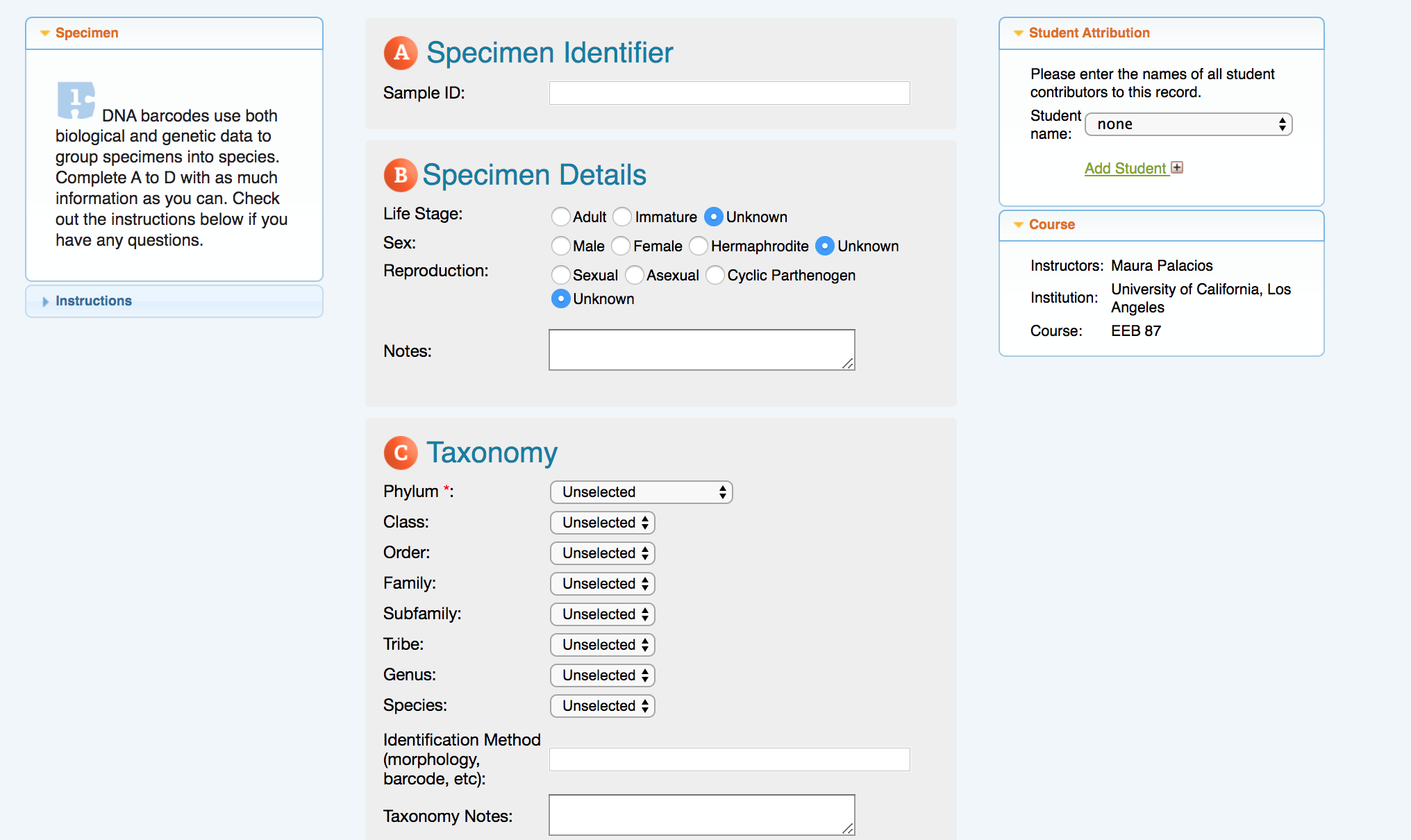
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For the login information, type in **class-878** for the username and **DTL77** as the password (Be sure to type in). This will take you the main console. We will be working in the data management console. **Click new specimen**

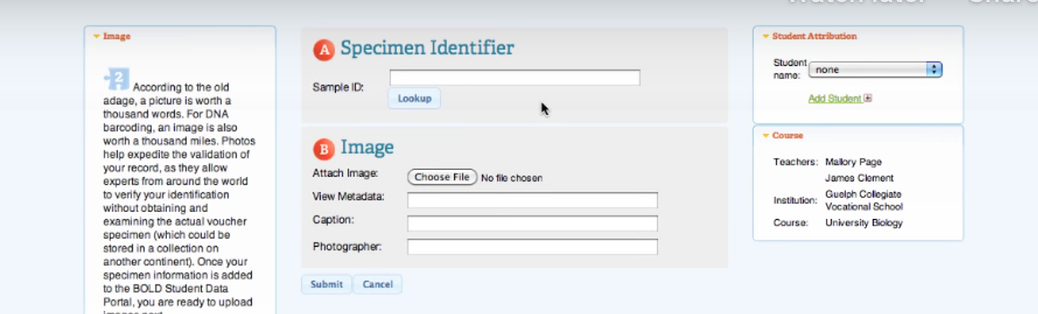


That will take you to this window. For Specimen voucher, we will be using the format year\_initialoftheschool\_yourinitials\_samplenumber. Some of you may have the larval or adult stage of the organism. Input the taxonomy from the GBIF or Genbank. Samples were collected on October 5th and the coordinates are as follows: **Lat:** 34.202856 **Lon:** -118.166464

**Accuracy:** 66m



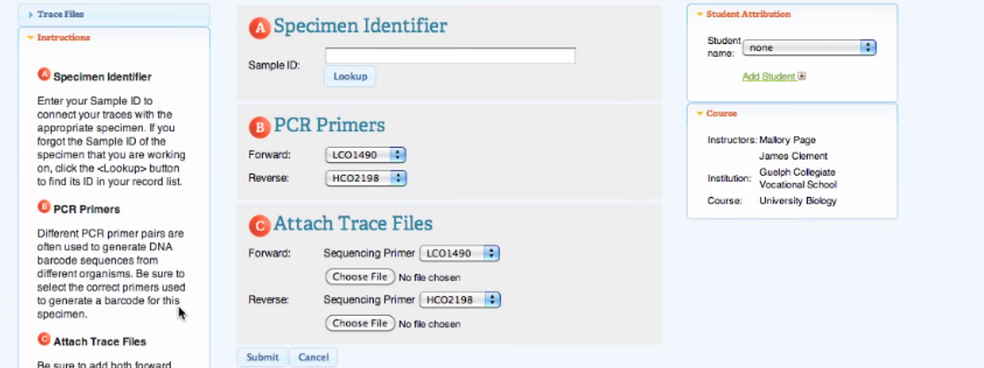
After you submit do not view record and you will be taken back to the data management console. **Click upload images and you will be taken to this screen.**



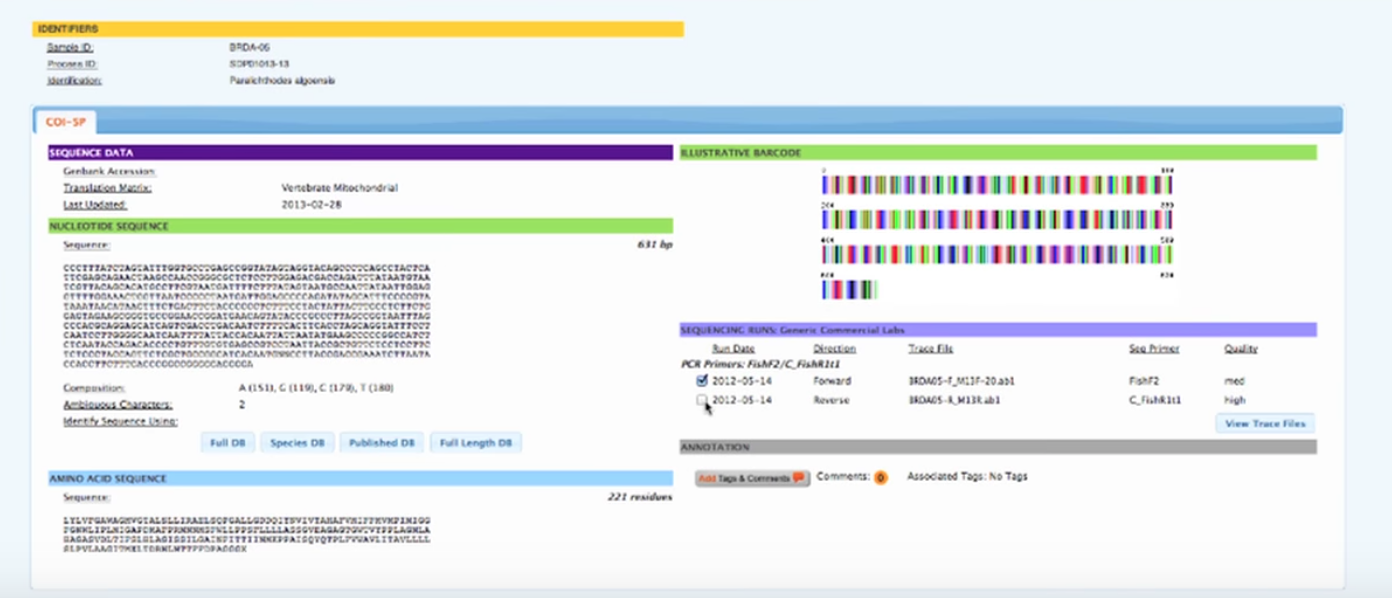
Search for the image of your specimen here (<https://drive.google.com/open?id=1hd3zupVR0cDNgtsxuLgaOXB0ebSbfJPC>) and upload. To change the view of the images, change from list to gridview. Download your image to desktop and then upload. Be sure to input **Caroline Schreck as the photographer.**

You can then view your submission or return to data management console. **From the main console click upload traces and you will be taken to this screen.**

Search for your trace files here (<https://drive.google.com/open?id=1DMMLlA2wHXGTRw2J8HDa29UCOgeJ05p2>) and upload corresponding sequence. Change view to list instead of gridview for easier access. Download your sequence files by clicking the three dots and select download to your Desktop and upload to the server. \* Note you may only have one sequence.



From the main console **click add sequence** and you will be taken to this screen. Follow the prompts to finalize sequence submission.



CONGRATULATIONS!!! You just submitted new data to the BOLD database.