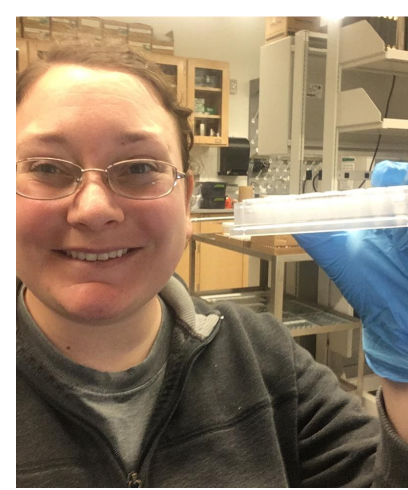


Great Lakes DNA Barcode Reference Library: Mollusca, Annelida, and Minor Phyla



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The Potential of DNA Barcoding



DNA Barcoding is the use of a short DNA fragment as a genetic tag for species identification



Early detection of species

Including invasive species, nuisance species, rare taxa, etc.



Increase the scope of biological surveillance



Allows taxonomists to focus reference specimens for establishing reliable databases versus routine identification (Valentini, Pompanon, and Taberlet 2009)

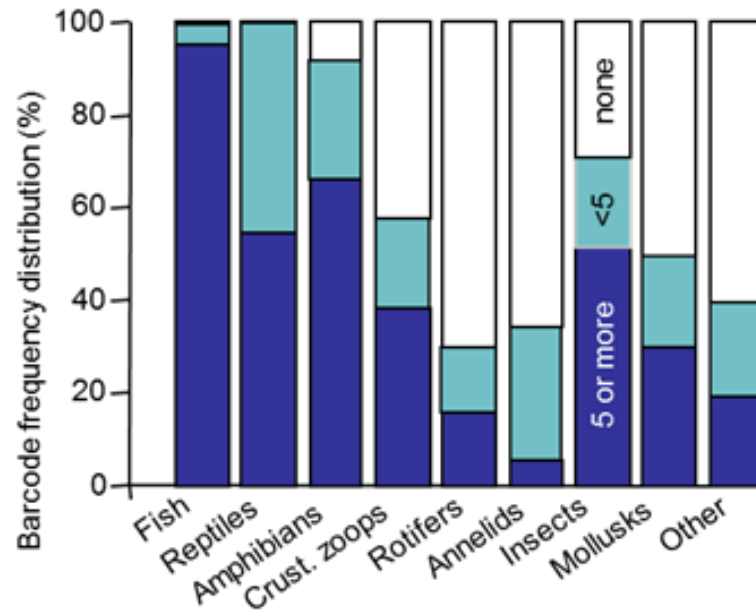


Helps distinguish species that are: (Decaëns et al. 2013)

Morphologically similar
High in phenotypic variability
Ambiguous juvenile stages
Damaged



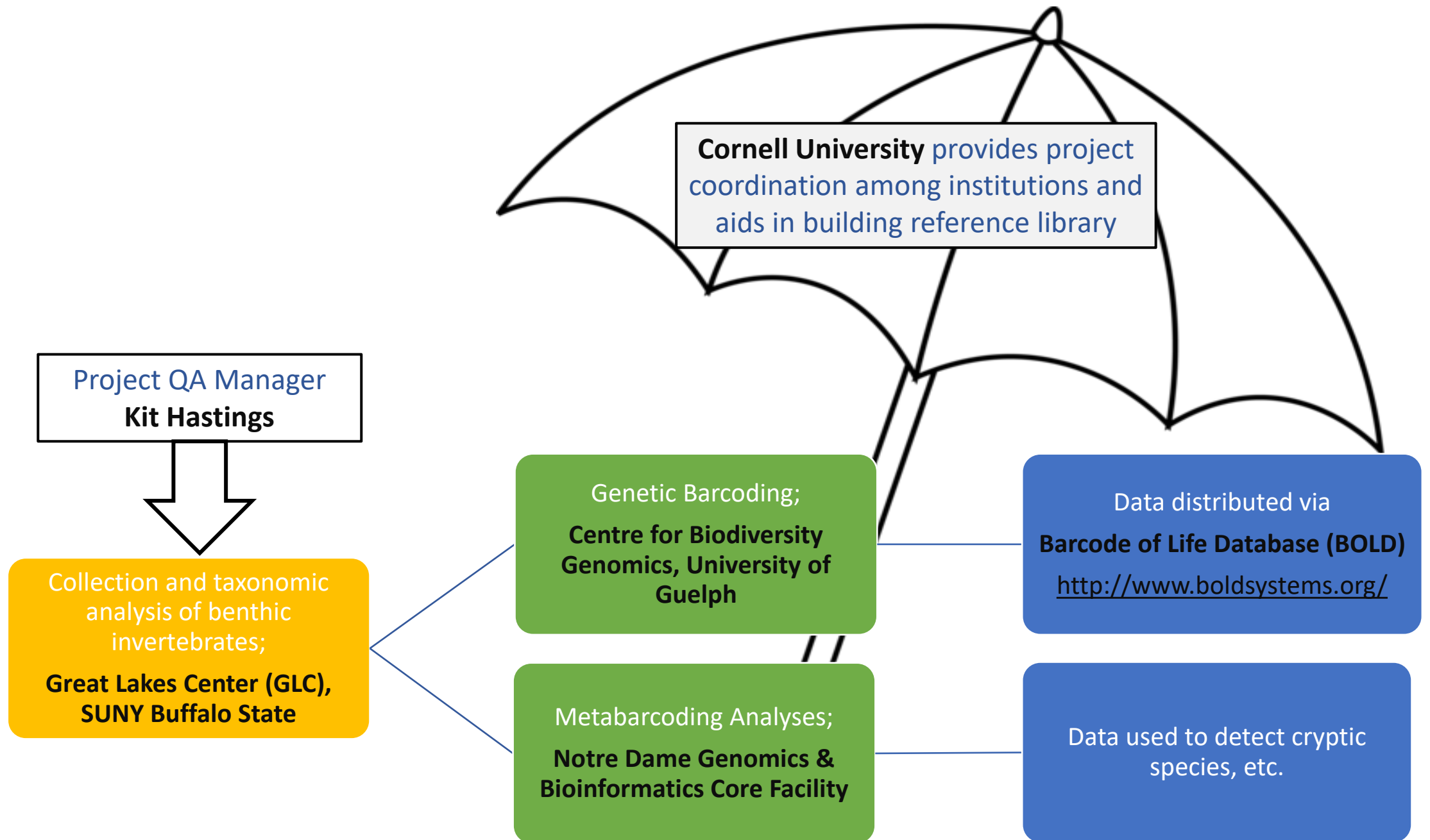
Project Goals



Trebitz et al. 2015 Sci. Rep. 5:12162.

- Generate voucher collection with high quality photos
- Clear up common taxonomic questions
- Identify locations with cryptic species to describe new species (Metabarcoding)
- Fill in the gaps of missing taxa in genetic barcode reference libraries (Genetic Barcoding)





In the Beginning

Phylum	Total by Trebitz (2015)	Synonyms	Valid	Still need barcodes	Percentage
Annelida	130	15	115	94	3%
Mollusca	148	8	140	76	46%
Bryozoan	7	0	7	7	0%
Cnidarian	3	0	3	2	33%
Kamptozoa	1	0	1	1	0%
Nematomorpha	1	0	1	1	0%
Nemertea	1	0	1	1	0%
Platyhelminthes	8	0	8	6	25%
Porifera	3	0	3	2	33%
TOTAL	302	23	279	190	32%





- Select GLNPO Long-Term Monitoring Stations (orange, n=27)
 - Nearshore stations (green, n=83)
 - Collaborative exchange of species (yellow and red, n=83)





Field Collection/Preservation

- Collected in 2017-2019
Spring/Summer/Fall
- Use nets, PONAR, and
qualitative methods
- Live picked, preserve in
200 proof ethanol,
undiluted, and place on
ice
- Replace ethanol after
24 hours, and stored in
freezer



Collaboration



Dr. Valerie Brady (University of Minnesota) shared ~400 specimens of Mollusca and Annelida (Great Lakes Coastal Wetland). In return, we shared over 1000 specimens



Graduate student Adam Frankiewicz (U Minnesota Duluth) collected and sent for genetic analysis 16 of the missing 17 species from the family Pisiidae.



Cornell teams (Lindsay Schaffner, James Watkins, Chris Marshal, Beth Whitmore, Joe Connolly and Lars Rudstam) shared specimens



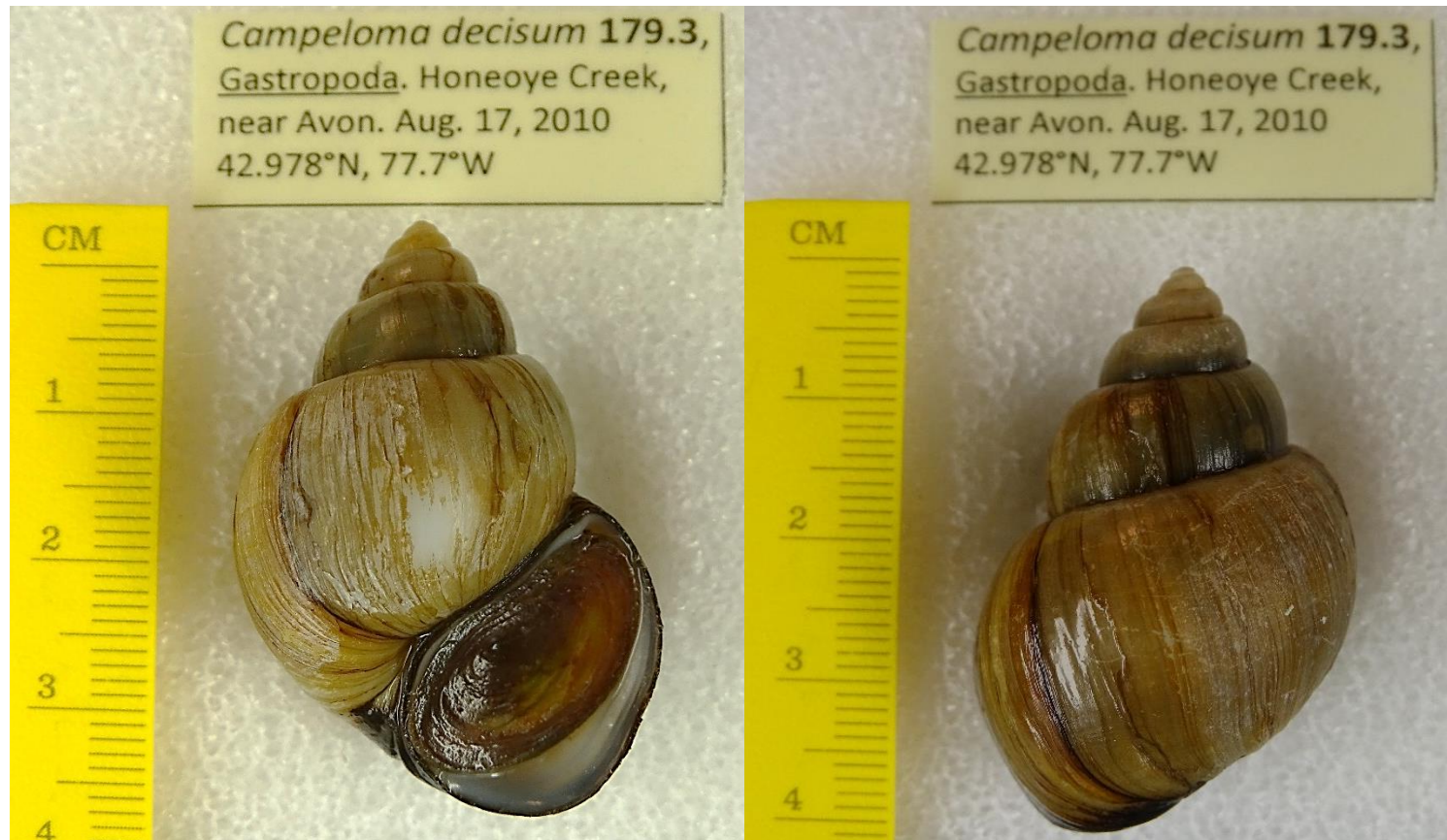
Mandi Ohar from U.S. FWS shared Annelida from over two years of river/embayment collections



Taxonomic Experts

- Ronald W. Griffiths, Oregon State University
 - General Taxonomy
- Nadine Rorem, Wheaton College
 - Cnidaria
- Arthur Bogan, NC Museum of Natural Sciences
 - Unionidae
- Timothy S. Wood, Bryo Technologies LLC
 - Bryozoa





Taxonomy and Classification

- Taxonomists use standard keys widely accepted in GL region (i.e. Kathman and Brinkhurst, 1998; Clarke, 1981; Jokinen, 1992; Smith and Pennak, 2001; Klemm, 1985)
- Most species are confirmed by an external taxonomist
- Photos are taken of individuals



Data Management

- Developed an Access database
- Automatically assigns species/individual number
- Contains information on species collection, identification by experts, attached photos, sample custody logs, etc.

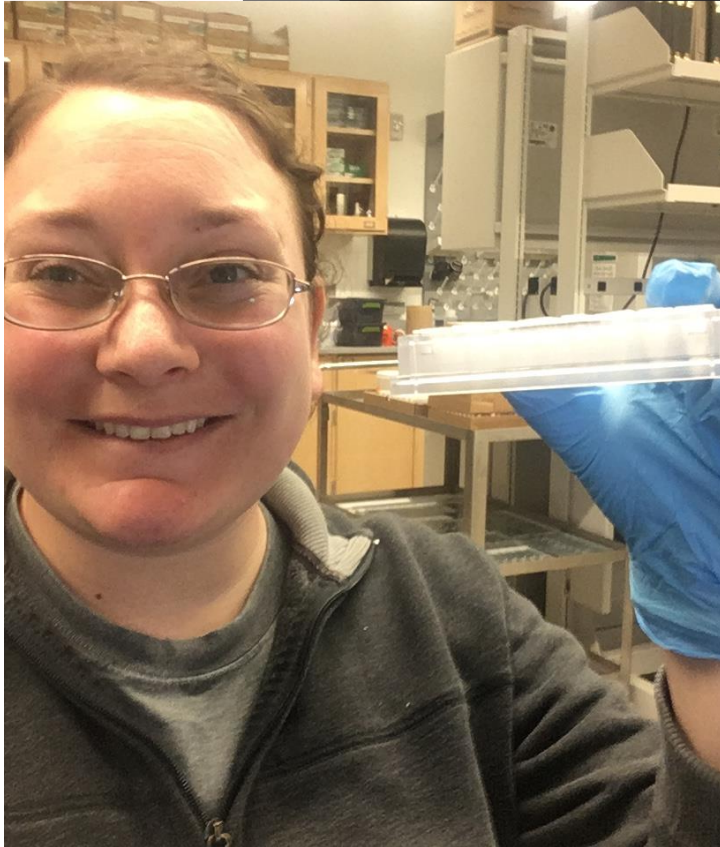
SUNY Buffalo State-G

ID	220	Species	Probythinella lacustris		
Class	Gastropoda	Order	Littorinimorpha	Family	Hydrobiidae
# Barcodes in BOLD	0	Species Completed	<input type="checkbox"/>	Date Species Completed	
Intitals					

Specimen (X1)	220.1	Specimen (X2)	220.2	Specimen (X3)	220.3
X1 Site	ER61	X2 Site	ER61	X3 Site	Lake Erie, western basin
X1 Lat. (N)	41.946667	X2 Lat. (N)	41.946667	X3 Lat. (N)	41.70582
X1 Long. (W)	-83.045	X2 Long. (W)	-83.045	X3 Long. (W)	-83.46254
X1 Habitat Details and Site Description	Lake Erie, Western Basin, 7.98m	X2 Habitat Details and Site Description	Lake Erie, Western Basin, 7.98m	X3 Habitat Details and Site Description	Maumee River Toledo
X1 Habitat Type	Lake Open Water	X2 Habitat Type	Lake Open Water	X3 Habitat Type	Lake Nearshore
X1 Collected By?	Susan Daniel	X2 Collected By?	Susan Daniel	X3 Collected By?	USFWS (M084)
X1 Collection Date	20170812	X2 Collection Date	20170812	X3 Collection Date	20140716
X1 Taxonomist	A. Karatayev	X2 Taxonomist	A. Karatayev	X3 Taxonomist	A. Karatayev
X1 ID Confirmed	R. Griffiths	X2 ID Confirmed	R. Griffiths	X3 ID Confirmed	R. Griffiths
X1 Date of ID Confirmation	20180417	X2 Date of ID Confirmation	20180418	X3 Date of ID Confirmation	20180418
X1 Sent for Barcoding	20180521	X2 Sent for Barcoding	20180521	X3 Sent for Barcoding	20180521
X1 Received Molecular Data		X2 Received Molecular Data		X3 Received Molecular Data	
X1 Specimen Fully Processed		X2 Specimen Fully Processed		X3 Specimen Fully Processed	
X1 Voucher?	No	X2 Voucher?	No	X3 Voucher?	No
X1 Plate #	CCDB-30668 A11	X2 Plate #	CCDB-30668 A12	X3 Plate #	CCDB-30668 B01
					and well Sent whole Ind

Record: 220 of 257 No Filter 220

Preparing microplate



- Taxa organized by primers and lysis buffers
- Submitting a small amount of tissue, or whole individual if < 3 mm
- 96 well-plate (12x8)
- BOLD is provided with information on:
 - sample location
 - collector(s)
 - sampling protocol
 - habitat type
 - taxonomist
 - life stage
 - unique identifiers

*Things are then shipped to BOLD, and magic happens

** Sequences- Cytochrome Oxidase I (COI)



Bold Systems v4

Not secure | www.boldsystems.org/index.php

SUNY Buffalo State Great Lakes Center... E. H. Butler Library... Google Scholar Macro Photograph... Bing Journal of Great La... Your Shopping Cart... Susie Databases En...

BOLD SYSTEMS

DATABASES IDENTIFICATION TAXONOMY WORKBENCH RESOURCES LOGIN

BARCODE OF LIFE DATA SYSTEM ^{v4}

Advancing biodiversity science through DNA-based species identification.

EXPLORE THE DATA

DESIGNED TO SUPPORT THE GENERATION & APPLICATION OF DNA BARCODE DATA

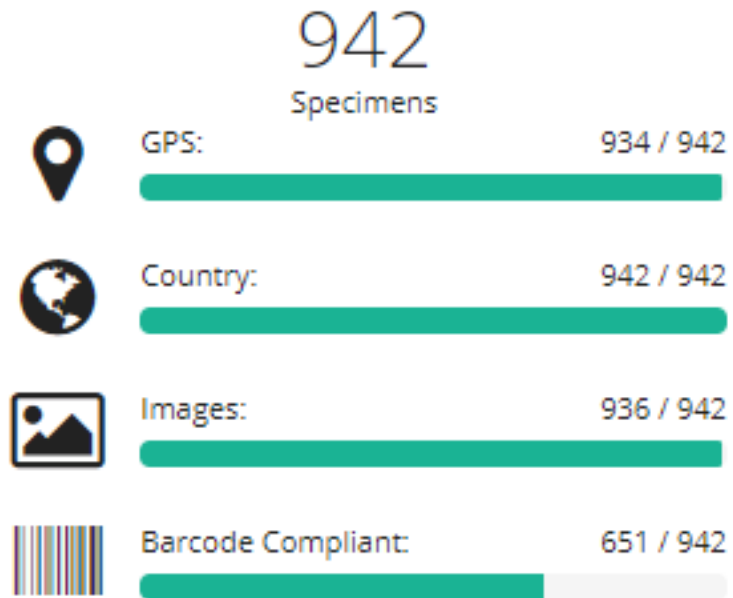
BOLD is a cloud-based data storage and analysis platform developed at the Centre for Biodiversity Genomics in Canada. It consists of four main modules, a data portal, an educational portal, a registry of BINs (putative species), and a data collection and analysis workbench.



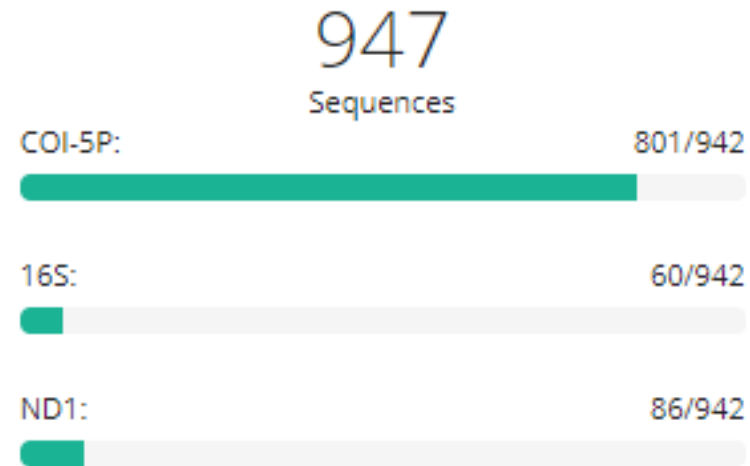
Phylum	Beginning	% target species barcoded in BOLD to date based on BSC submission	World-wide % barcoded target taxa May 2020
Annelida	3%	43.5%	55.7%
Mollusca	46%	87.1%	87.1%
Bryozoan	0%	28.6%	71.4%
Cnidarian	33%	100%	100%
Kamptozoa	0%	0%	0%
Nematomorpha	0%	100%	100%
Nemertea	0%	100%	100%
Platyhelminthes	25%	62.5%	62.5%
Porifera	33%	33.3%	33.3%



Specimens

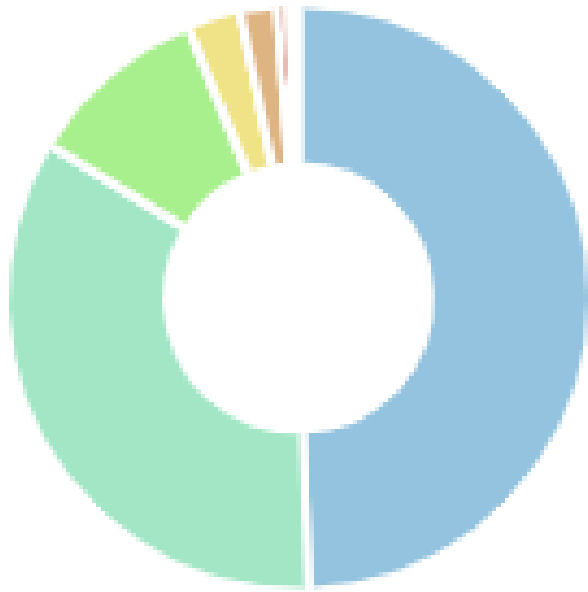









Sequences



- 13 genetic microplates submitted (96 wells)
 - some partial plates
- 942 total specimens
- 801 yielded sequences (85%)
- 651 reached barcode compliance (69%)



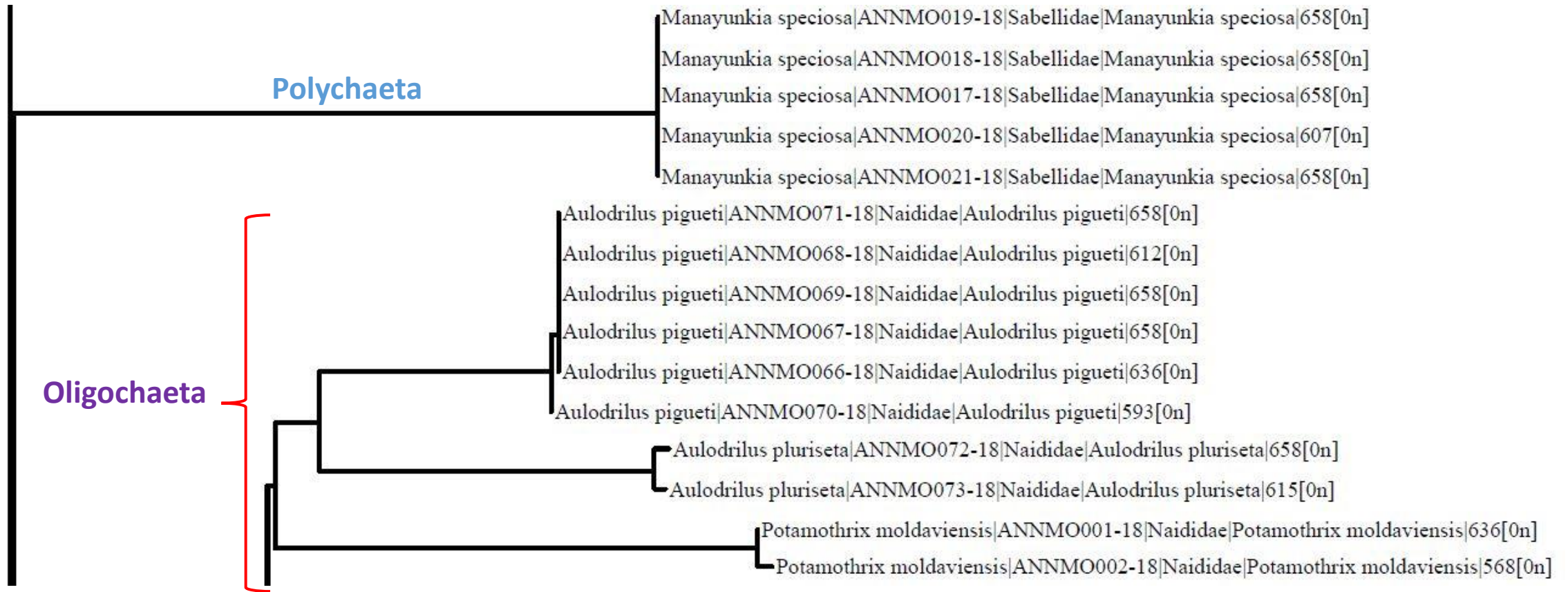


	Mollusca (phylum): 466
	Annelida (phylum): 323
	Cnidaria (phylum): 95
	Platyhelminthes (phylum): 28
	Bryozoa (phylum): 19
	Nematomorpha (phylum): 6
	Nemertea (phylum): 5

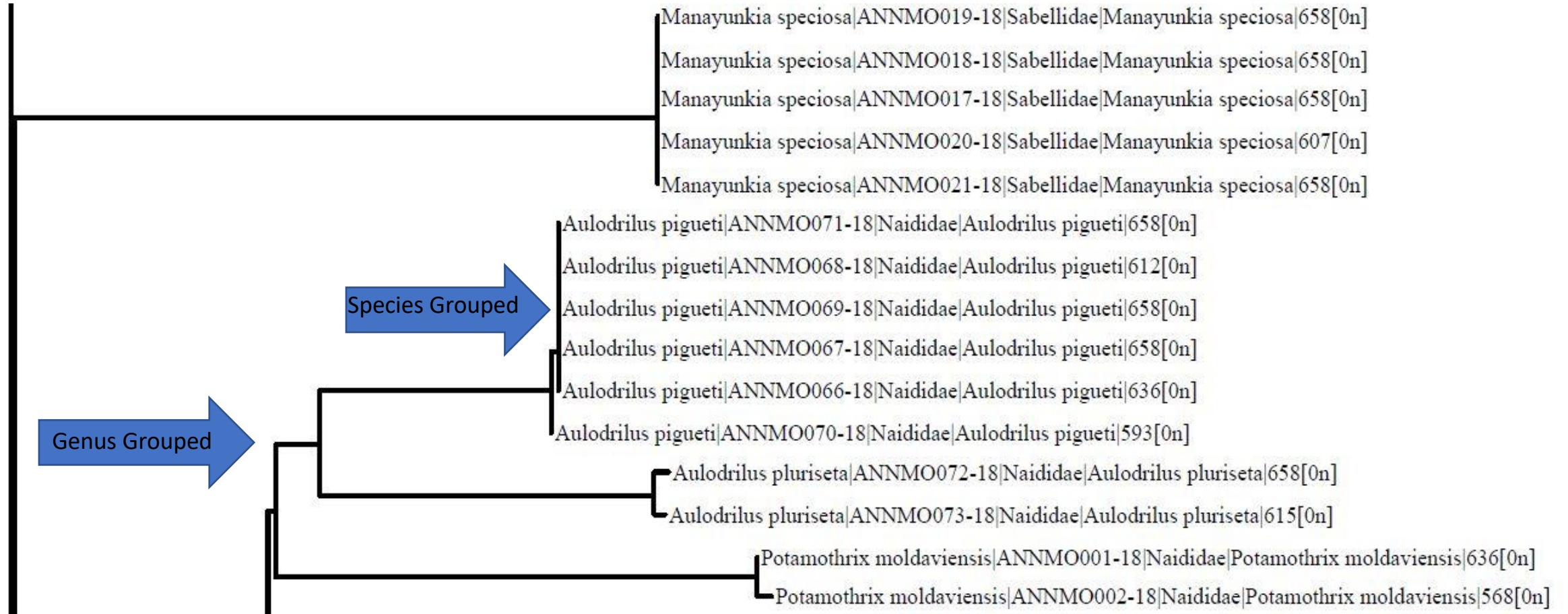
Submitted Specimens by Phylum



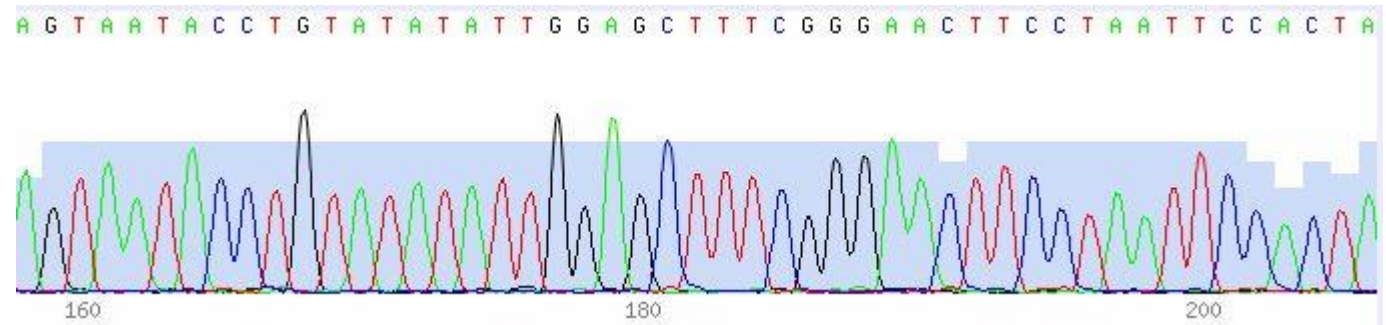
Sequencing Results Highlight



Sequencing Results Highlight

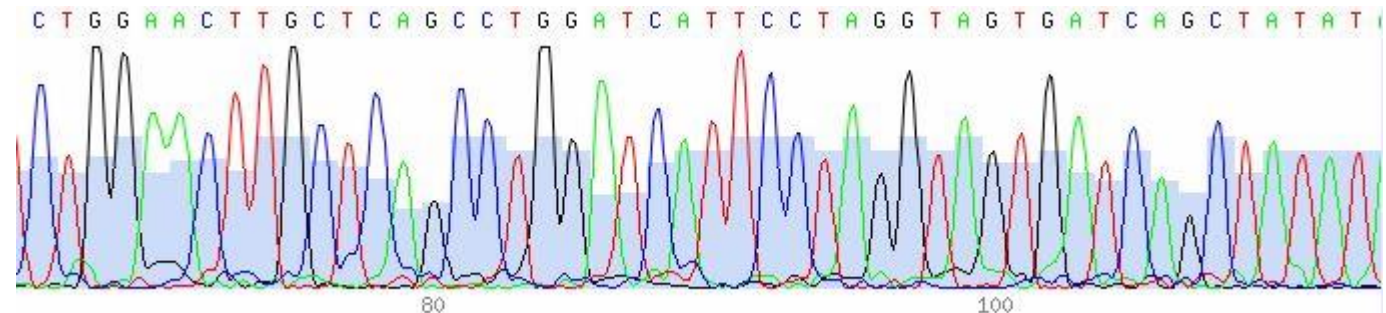


The Good



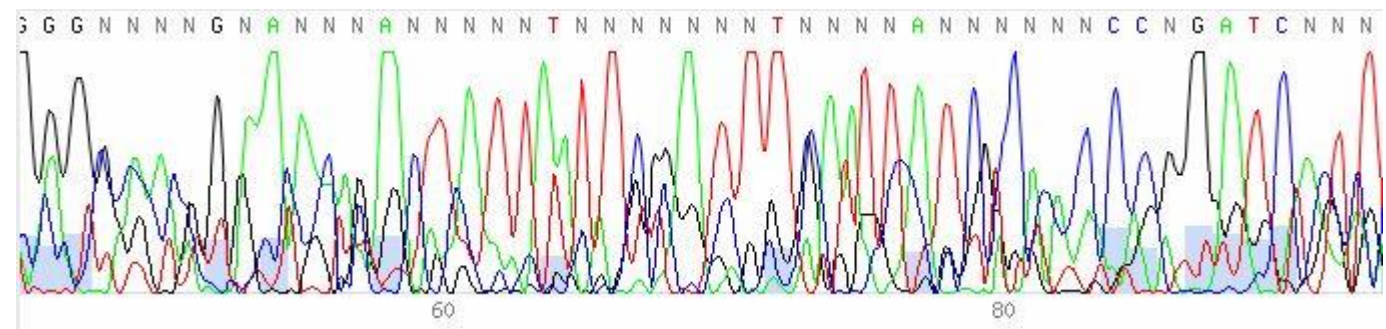
Potamothrix moldaviensis, high quality sequence

The Bad



Potamothrix moldaviensis, medium to low quality sequence

The Ugly



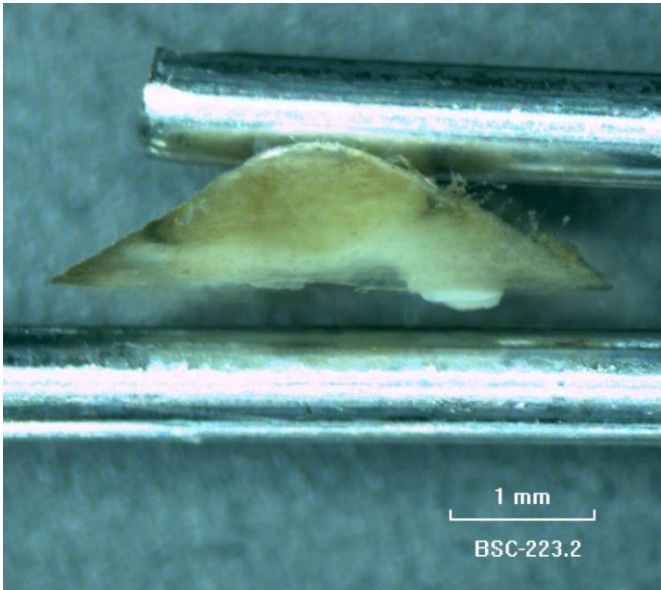
Limnodrilus claparedianus, co-amplification, failed quality



Continued Work

- Verifying taxonomy in BOLD
- Flagging problematic taxa (see next slide)
- Finalizing Great Lakes Barcoding Reference Library
 - Including an up-to-date list of known species (aquatic invertebrates) within the basin
- Metagenomic sample processing and analysis





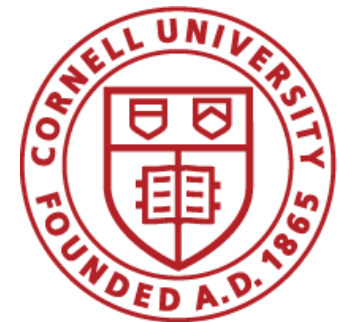
Challenges

- Handling small, delicate specimens
- Working around the operculum
- Immature oligochaete
- Licensing information for photos
- Props to orient specimens properly... a paperclip can do wonders!



Acknowledgements

- Funding: **U.S. EPA, project "DNA Barcode Reference Library: Mollusca, Annelida, and minor phyla"** Great Lakes National Program Office (GLNPO)
- US EPA Research Vessel Lake Guardian-Captain and crew
- All my authors and collaborators on this project
 - Jakob Boehler, Ron Griffiths, Jim Watkins, Lars Rudstam, Kristy Deiner, Lindsay Schaffner, Isabel Hannes, etc. there are so many!
- GLC staff (Kit Hastings, Erik Hartnett, Susan Dickinson, and Brianne Tulumello) and students
- Valerie Brady, Adam Frankiewicz and their collaborators
- Mandi Ohar, U.S. FWS



MVP



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