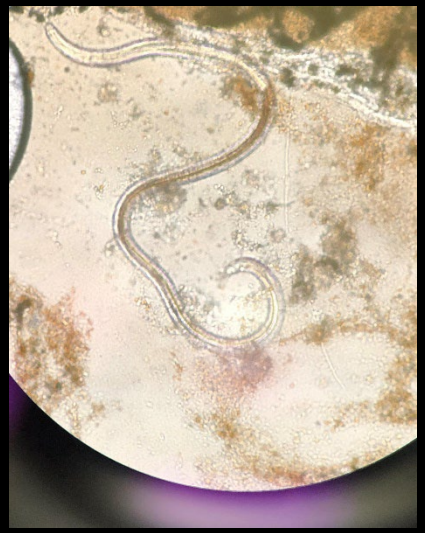
Trumpet snails



Larval Nematode

Larval Fluke

Giant Apple Snails

Surveying Freshwater Invasive Snails for Detecting Invasive Helminth Pathogens

Lori Tolley-Jordan

CENTRE COLLEGE

KING'S College LONDON

JSU JACKSONVILLE STATE UNIVERSITY

TROY UNIVERSITY 1887

GULF STATES MARINE FISHERIES COMMISSION

Surveying invasive snails in Florida- a case study

ANS – develop and implement technology and/or approaches to establish an early detection/rapid response program, prevent the introduction, slow the spread, eradicate, and/or control ANS in the Southeast US, Commonwealth of Puerto Rico, and US Virgin Islands.

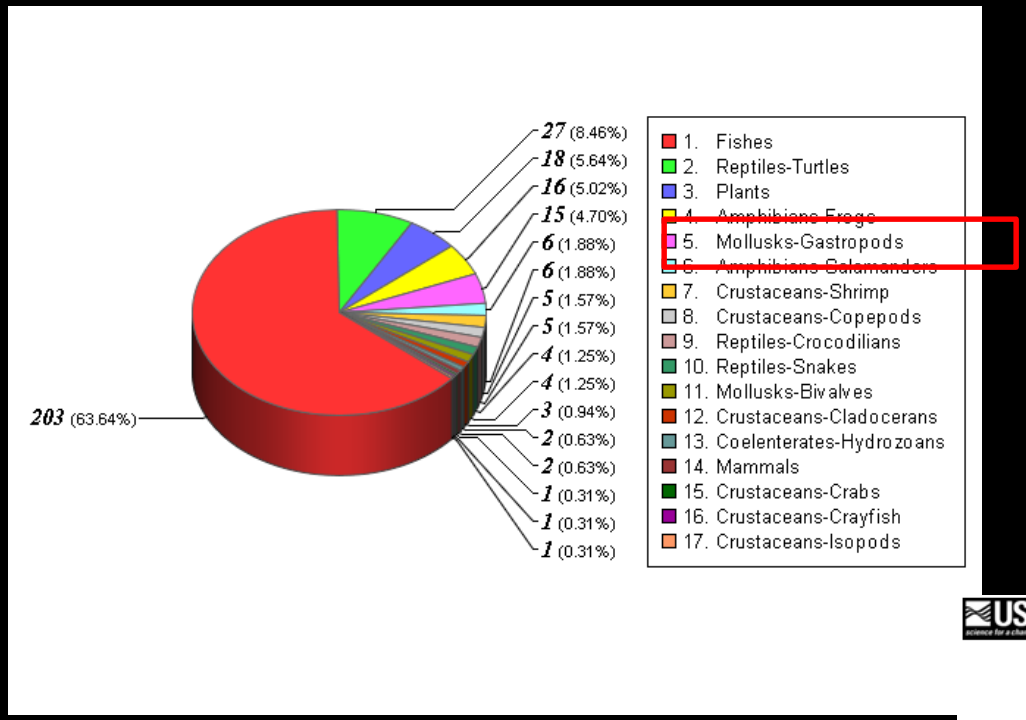
Increase systematic work on ANS that are difficult to Identify.

DETECT AND IDENTIFY HELMINTH PATHOGENS

- Small Size
- Complex Life Cycles

Restore or improve the ecological balance in habitats negatively affected by non-indigenous invasive or problem species in SARP's Southeast Aquatic Habitat Plan.

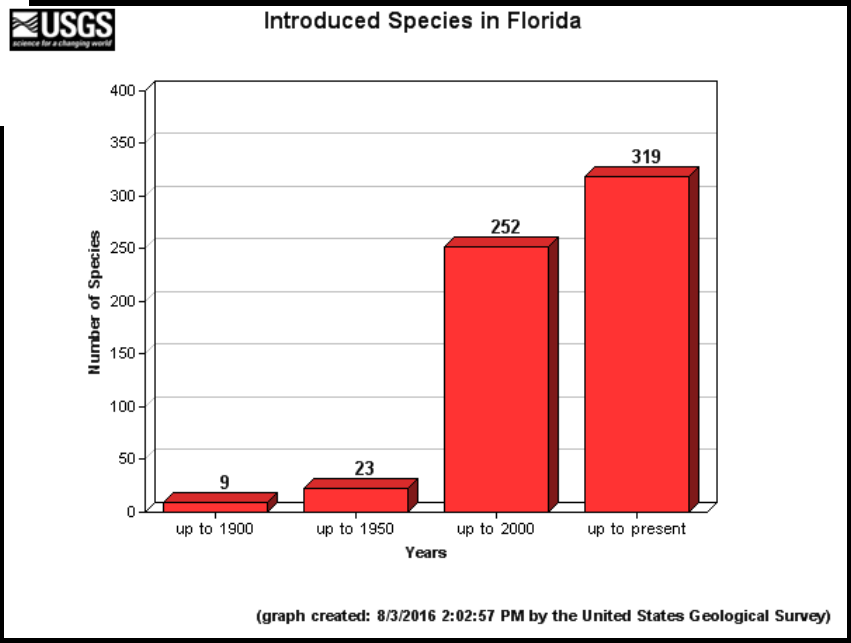
Why Florida?



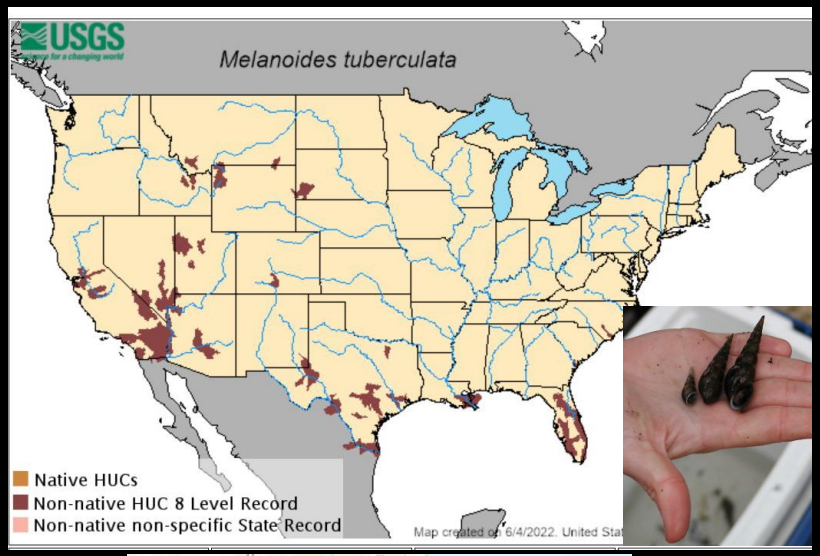
15 introduced snails

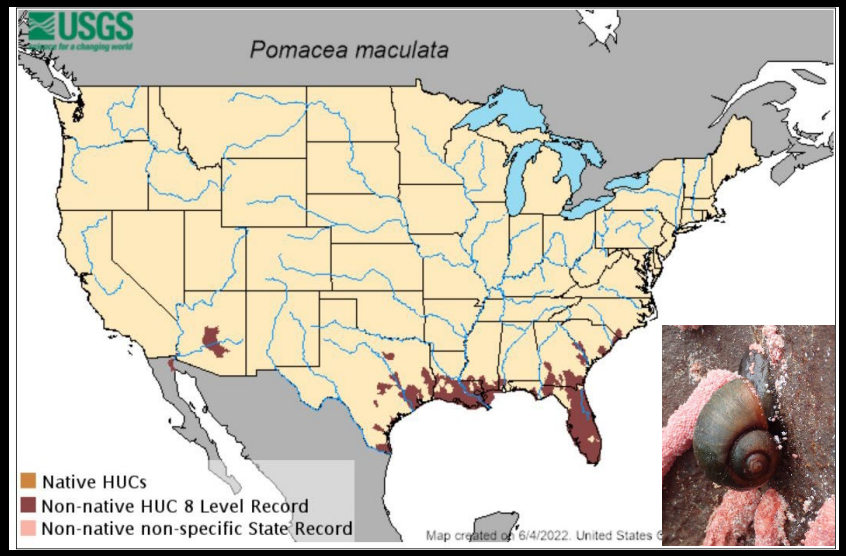
Most are aquatic
http://nas.er.usgs.gov/graphs/State.aspx

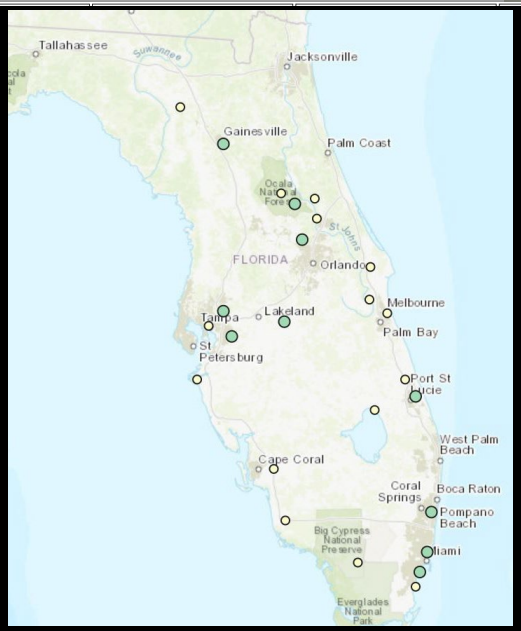
Helminth Pathogens are not on the list.

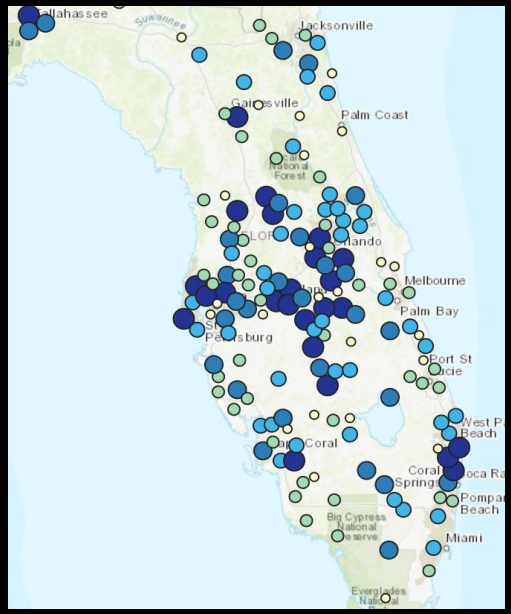


Invasive snails in Florida's Freshwaters

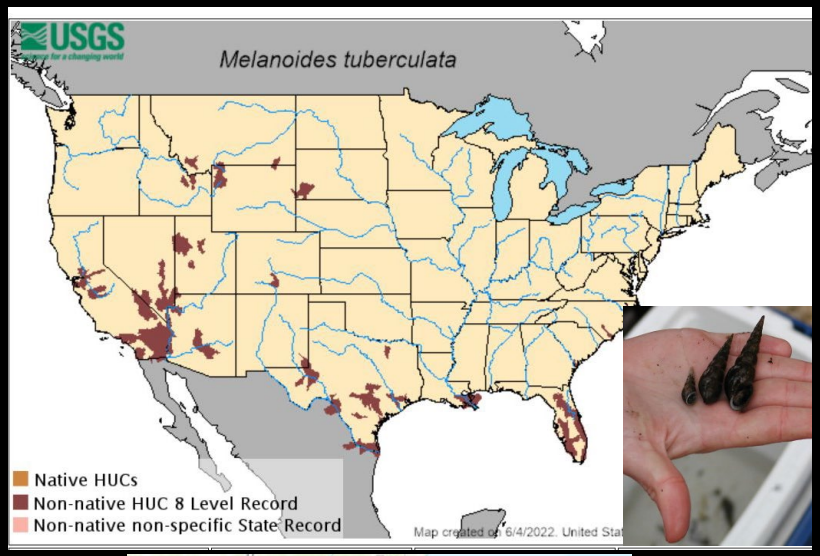


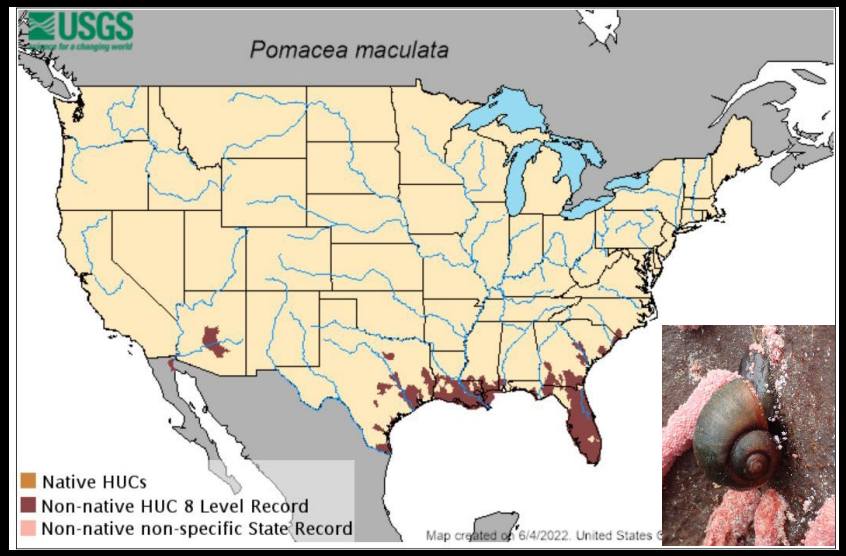


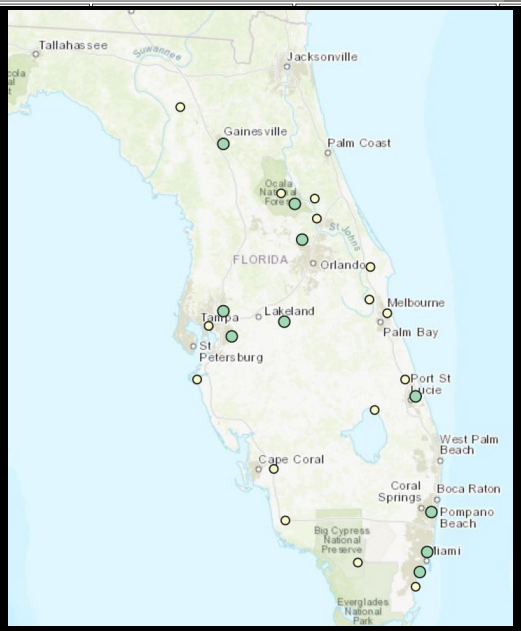


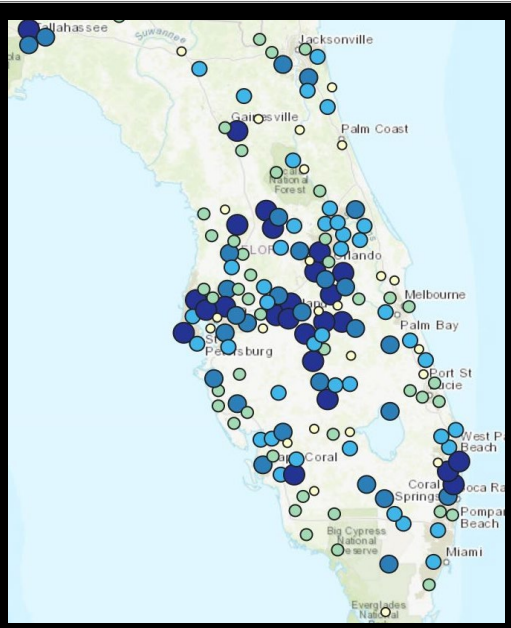


Who are their parasites?





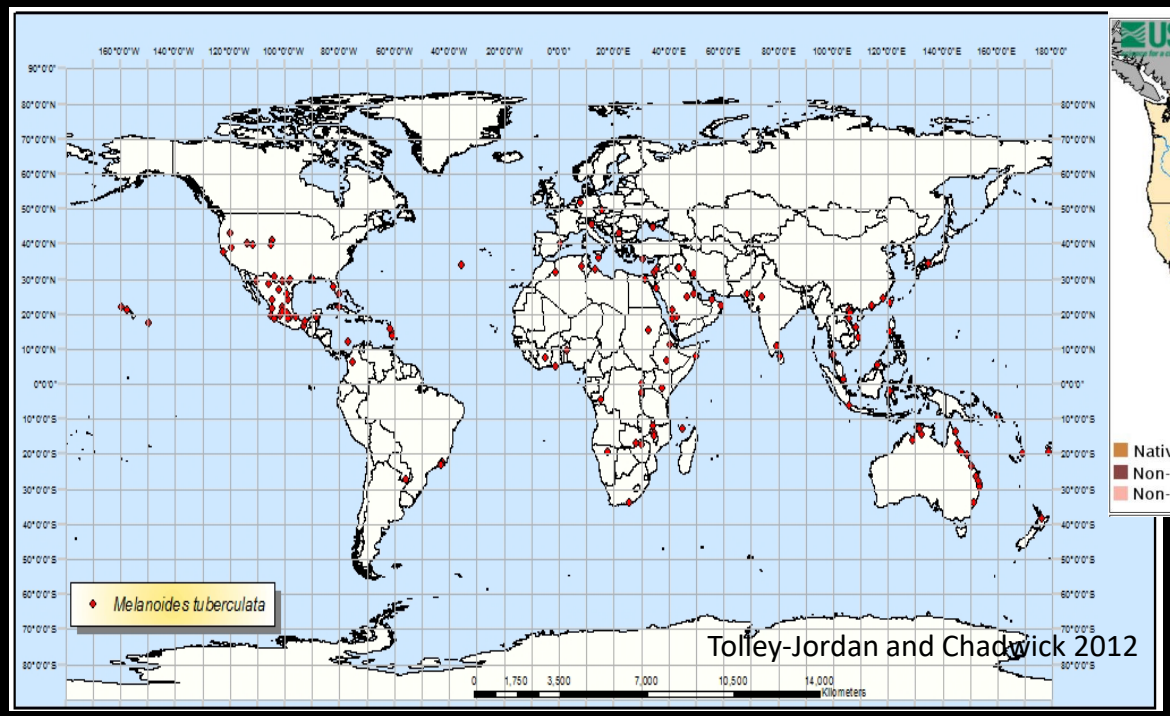




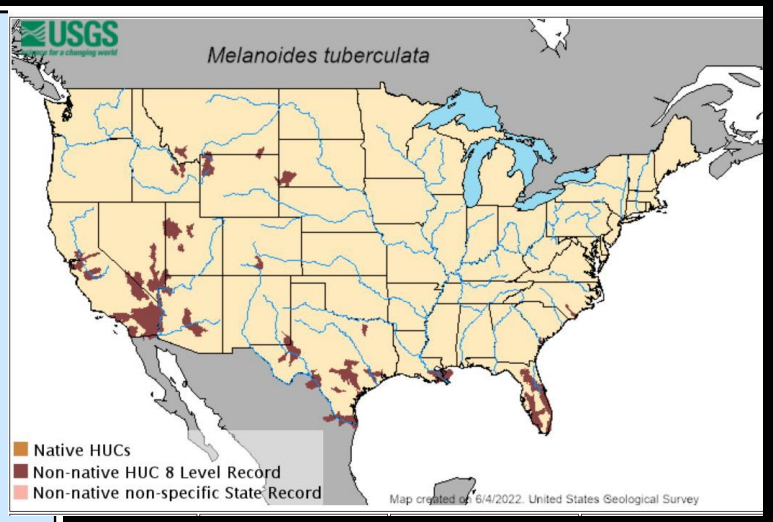
Study 1: 2016-2017

Study 2: 2019*-2021

Background: Distribution of trumpet snails- *M. tuberculata*



Tolley-Jordan and Chadwick 2012

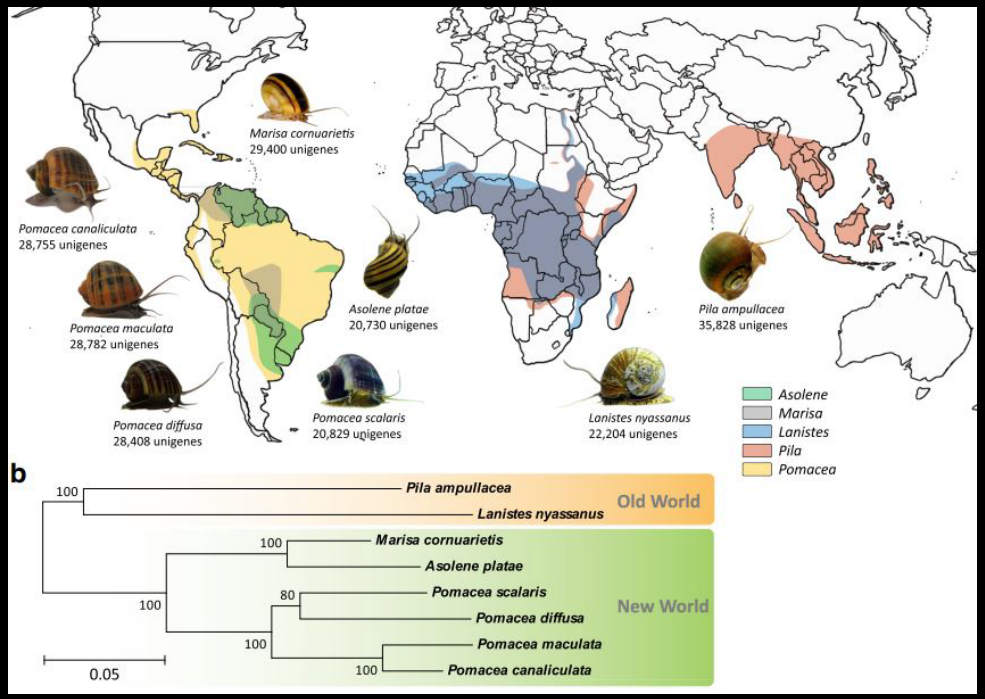


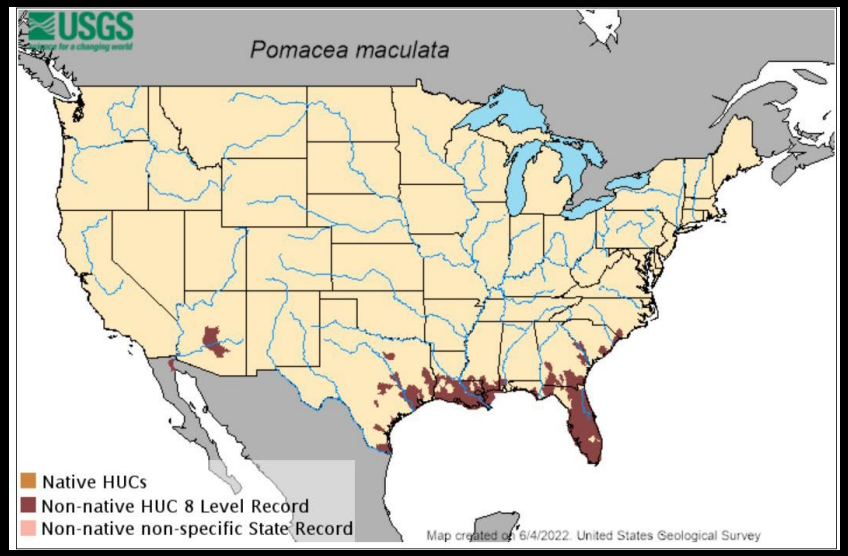
Thiaridae ~ 135 species

Obligate gill breathing snails

Global distribution in Tropical Freshwater/Brackish Waters

Background: Distribution of giant apple snails- *P. maculata*





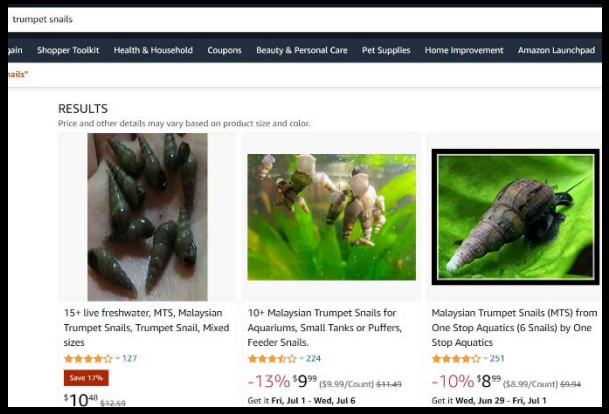
Ip, Jack CH, et al. "AmpuBase: a transcriptome database for eight species of apple snails (Gastropoda: Ampullariidae)." *BMC genomics* 19.1 (2018): 1-9.

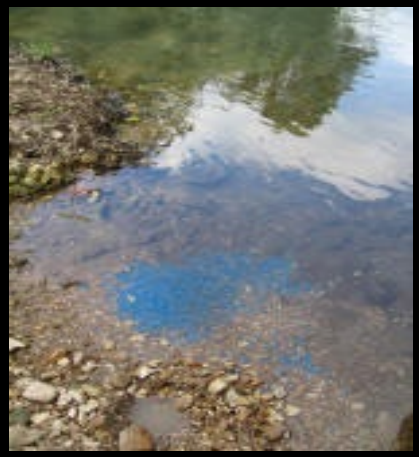
Ampullaridae ~ 7 species

Dual Respiration

Why are these snails in Florida?

Popular Aquarium Snails





Snail Traits for successful Invasion

Desiccation Resistant

High Salinity Tolerances

Large temperature range (15˚- 34˚C)

Large pH range

What makes *M. tuberculata* so successful?

Traits to Persist

- Female clonal
- Live Bearers
- High fecundity/iteroparous
- Generalist feeders

Problems

- Economic- ?
- Ecological- Competition
- Health- helminth pathogens

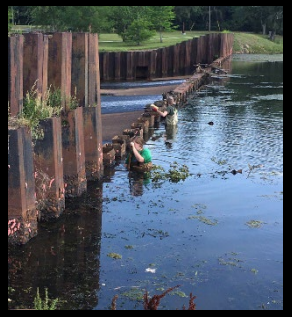
What makes *P. maculata* so successful?

Traits to Persist

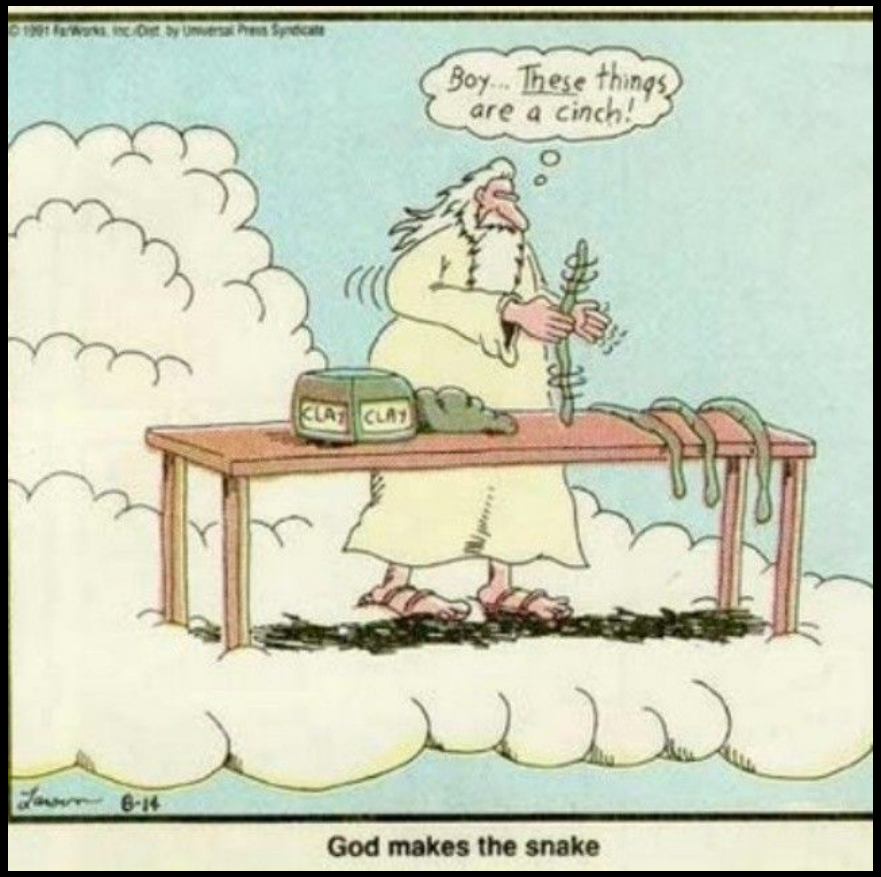
- Large Size
- Toxic eggs above waterline
- High fecundity/iteroparous
- Dual Respiration

Problems

- Economic- Ag pest
- Ecological- Competition
- Health- helminth pathogens



Helminth “worms”



Most common form in Animalia

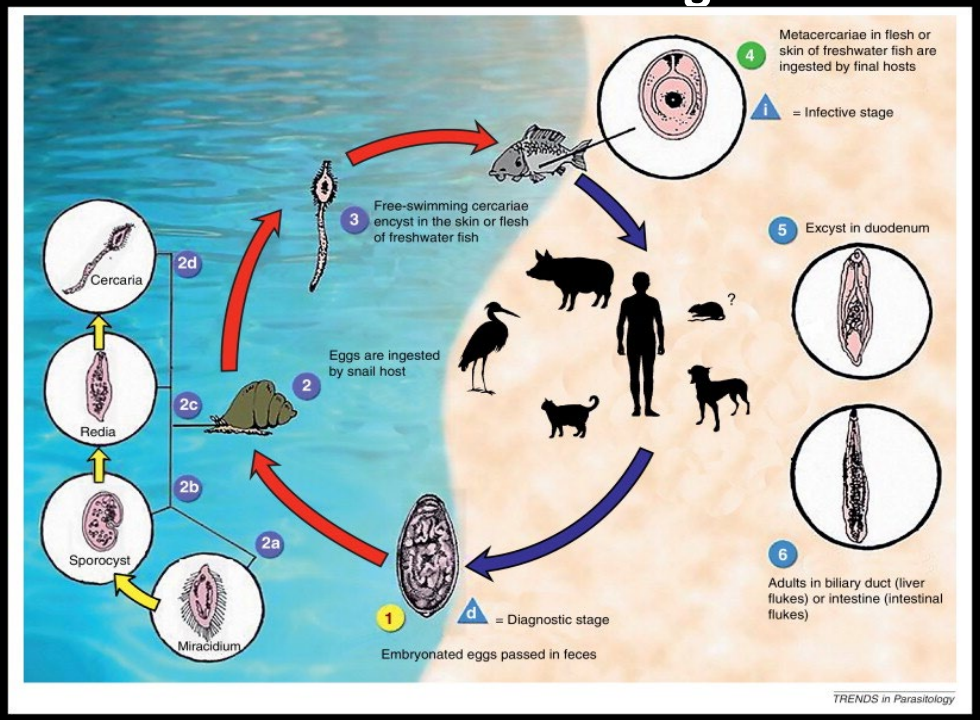
Flatworms

Non-Parasitic
Distant
Cousins

Roundworms

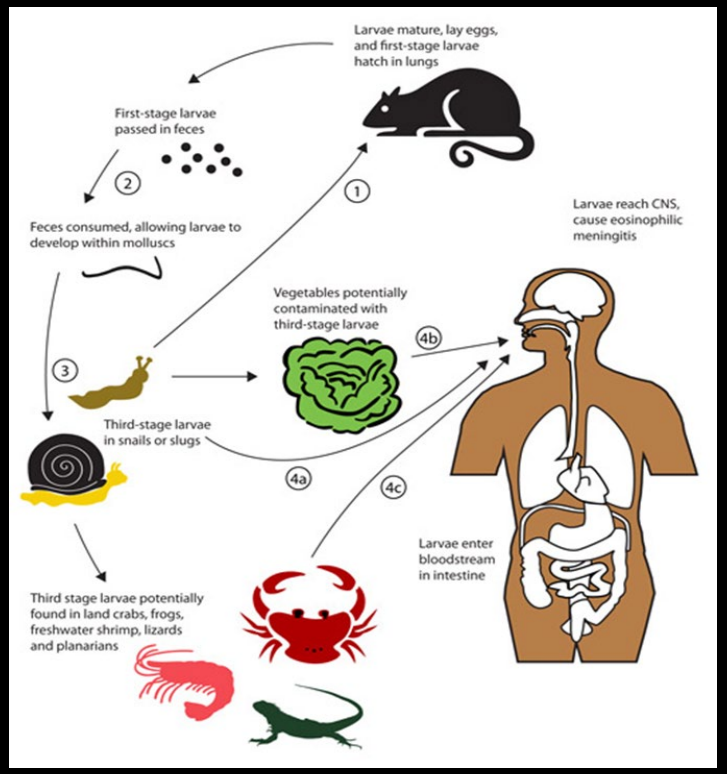
Snail-Helminth Relationships

Parasitic- Trematoda- Digenea



- Obligate Parasites in multiple hosts
- Parasite Host specific for intermediate host- Snail-site of asexual reproduction

Parasitic- Nematoda- Rhabditida



- Obligate Parasites in multiple hosts
- Parasite Host specific for definitive host- vertebrate

M. tuberculata trematodes in US prior to our survey

Native and Migratory Birds: TX, LA, AZ; 1970s

Philophthalmus gralli - Eye Fluke

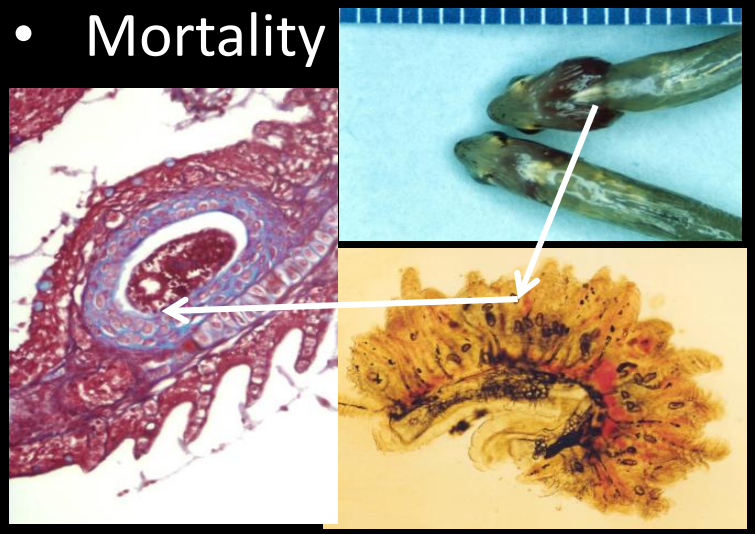
Pathology: Blindness

Church, et al. 2013. Veterinary Opthamology

Native Fishes: TX; 1990s

Centrocestus formosanus

- Respiratory stress
- Altered behavior
- Mortality

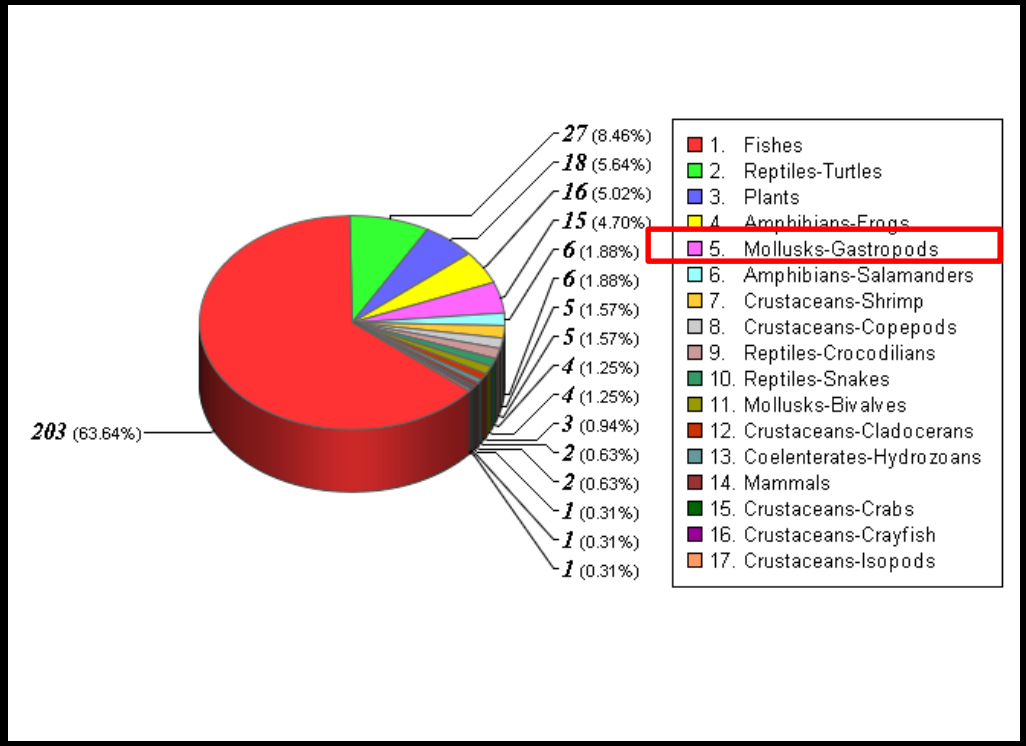


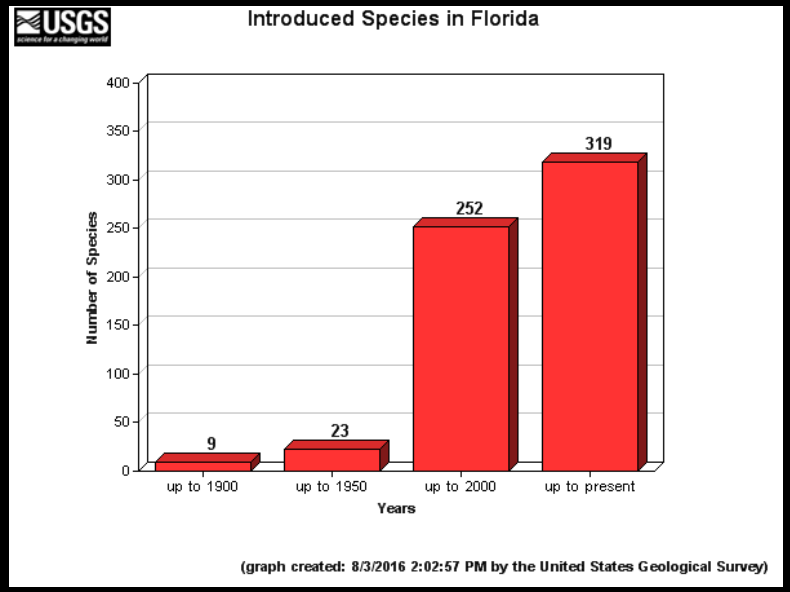
Native Fishes: TX; 2000s

Haplorchis pumilio

- Muscle Deformities
- Altered behavior
- Mortality

And many more possibilities for Florida





http://nas.er.usgs.gov/graphs/State.aspx

Zootaxa 2799: 15–28 (2011)
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

A checklist of trematodes (Platyhelminthes) transmitted by *Melanoides tuberculata* (Mollusca: Thiaridae)

HUDSON ALVES PINTO[1] & ALAN LANE DE MELO[2]

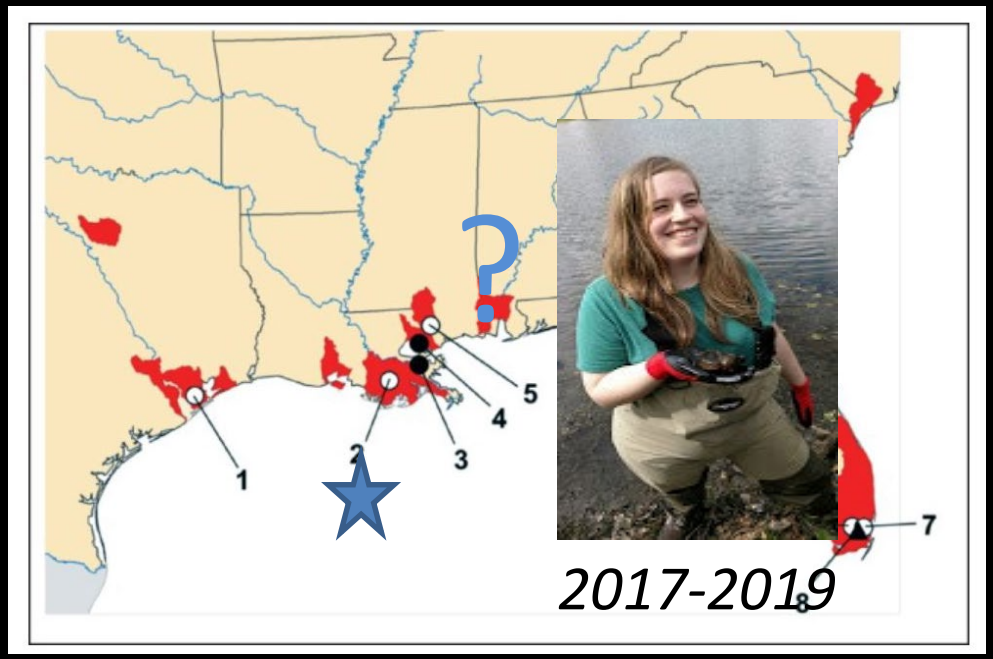
47 species

Apple snails infected with Nematodes in US prior to Survey

Hawaii J Med Public Health. 2013 Jun; 72(6 Suppl 2): 11–14.
PMCID: PMC3689474
PMID: 23901374

The Occurrence of the Rat Lungworm, *Angiostrongylus cantonensis*, in Nonindigenous Snails in the Gulf of Mexico Region of the United States

John L Teem, PhD, Yvonne Qvarnstrom, PhD, Henry S Bishop, BS, Alexandre J da Silva, PhD, Jacoby Carter, PhD, Jodi White-Mclean, PhD, and Trevor Smith, PhD



Nematoda sp.

Digenea sp.

Questions of surveys

- Do *M.tuberculata* populations in FL harbor trematode parasites?
- Who are the parasites?

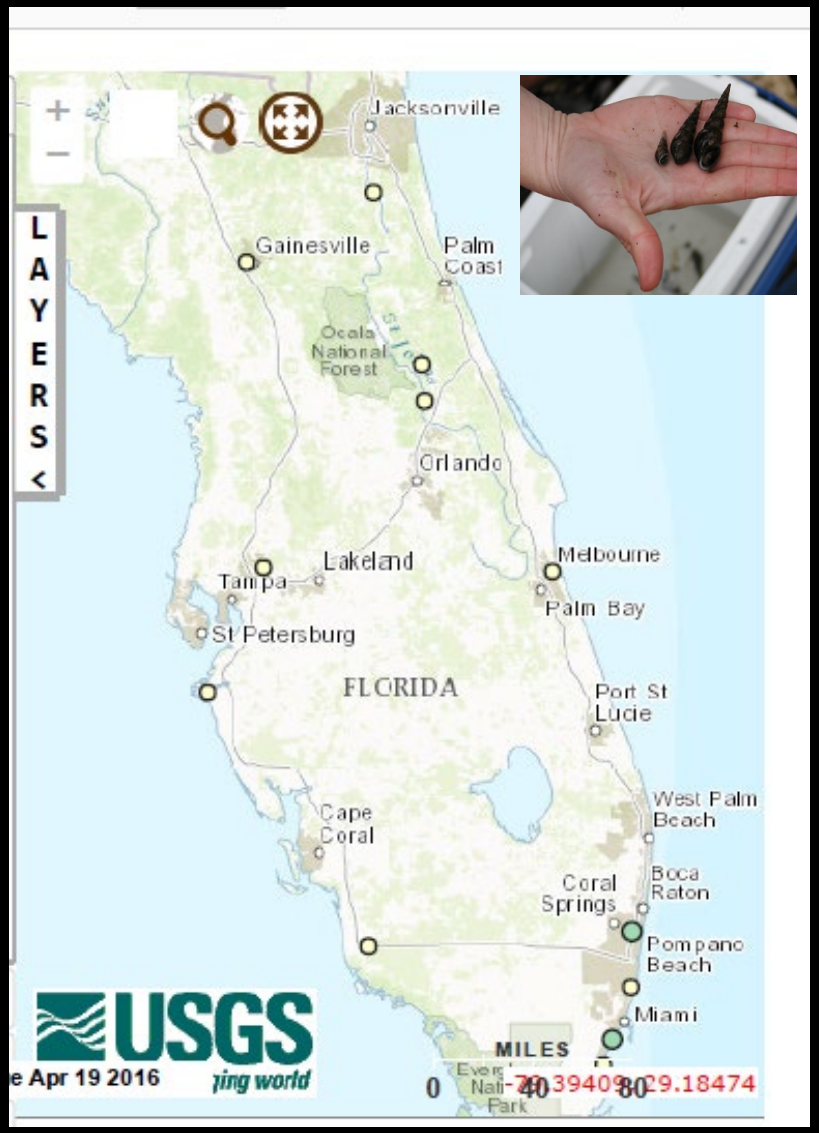
- Do *P. maculata* populations in FL harbor trematode parasites?
- Who are the parasites?

Questions

- Do *M.tuberculata* populations in FL harbor trematode parasites?
- Who are the parasites?

- Do *P. maculata* populations in FL harbor trematode parasites?
- Who are the parasites?

Methods: Finding the snails.

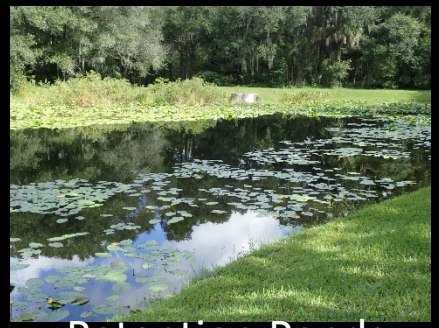


Streams

Open Water/Lakes

Canals/Ditches

Rivers



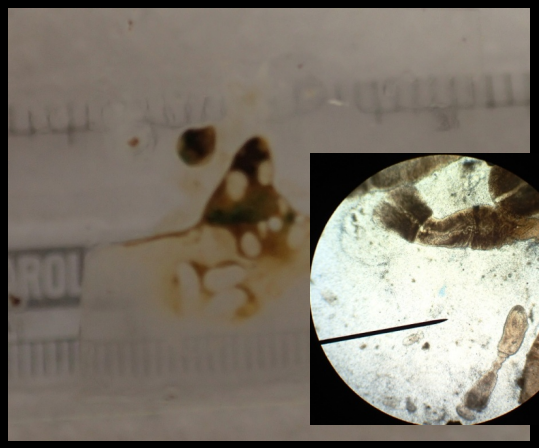
Retention Ponds

September 2016, 12 days,
2500 miles, 25 sites

Methods- Parasite Detection

Snails brought to "lab" live, in stream water. Each individual measured and sexed

Snails cracked, tissues removed

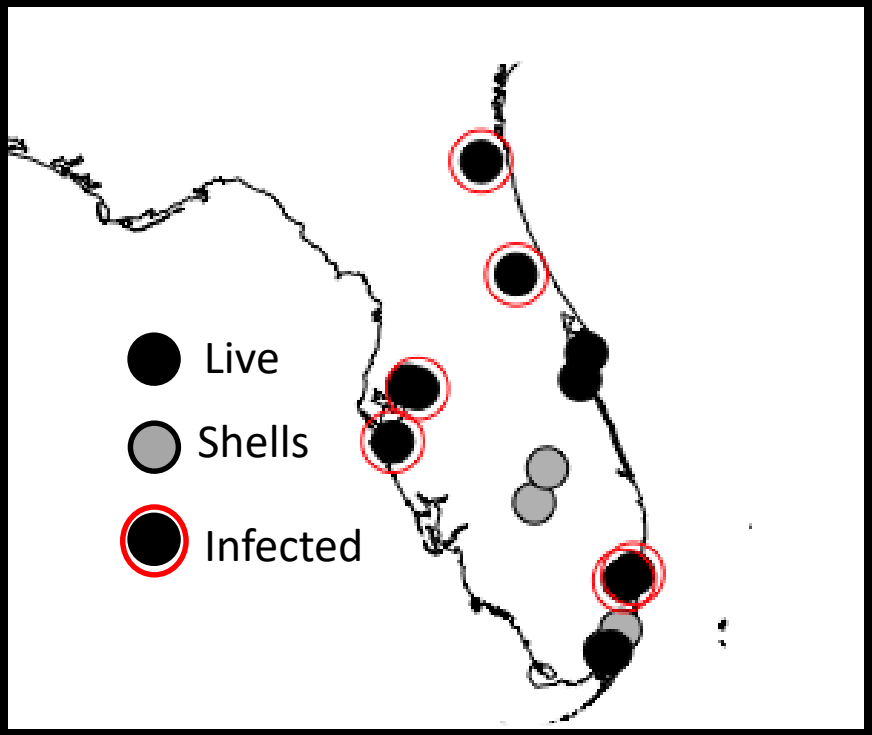


Tissue smear, observed under compound microscope

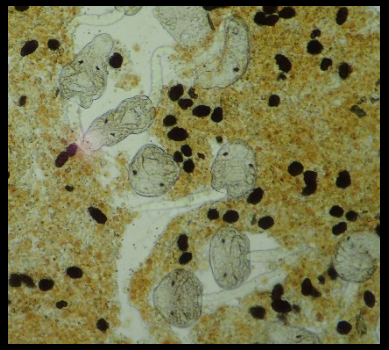
- Dissected ~ 300 snails

Results: Snails-yes & trematodes Yes

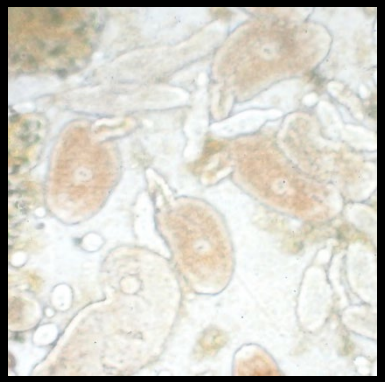
- 13 sites, live snails
- 7 sites, shells observed
- 6 sites, infected snails
- 5 trem spp.: 1-3 morphs per site



no eyes, single tail, stylet

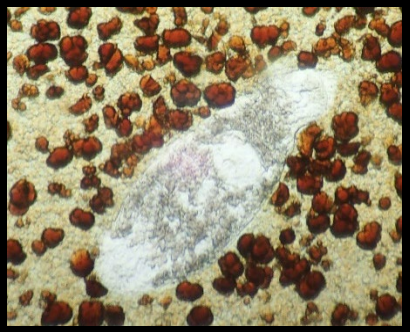


eyes, single tail, short



No eyes, single tail, short

eyes, single tail, long

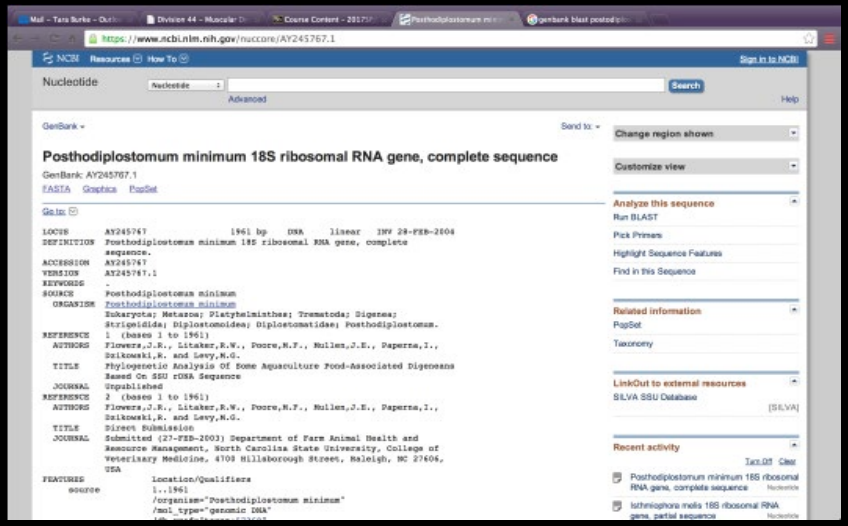


no eyes, peanut

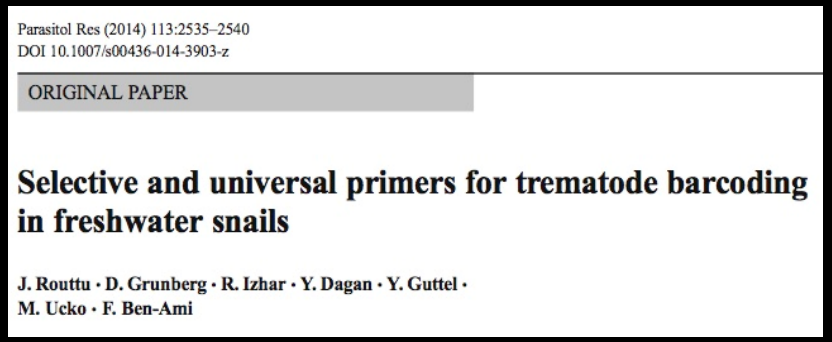
Methods- Parasite Identification

- Forty snails infected
- Snail tissues + Parasites preserved in 95% ETOH in Florida

- Samples sent to Jessica Wooten, Centre College, KY.
  - DNA extraction
  - DNA amplification
  - DNA sequencing



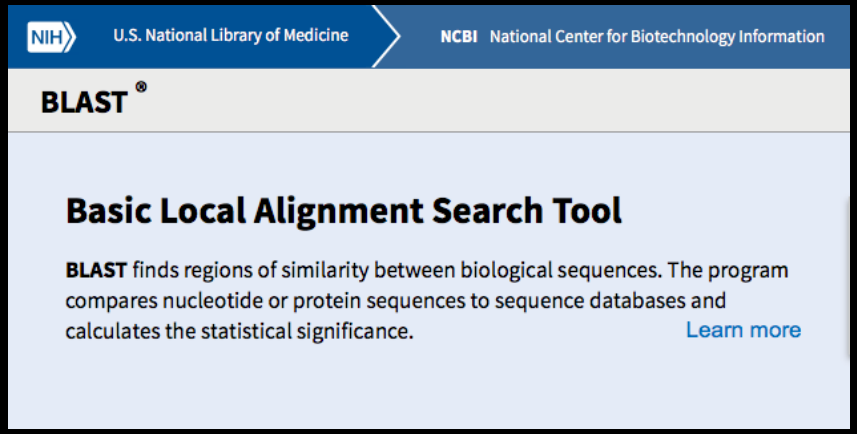
Closest % match of unknown to accessioned sequence in Genbank


Parasitol Res (2014) 113:2535–2540
DOI 10.1007/s00436-014-3903-z

ORIGINAL PAPER

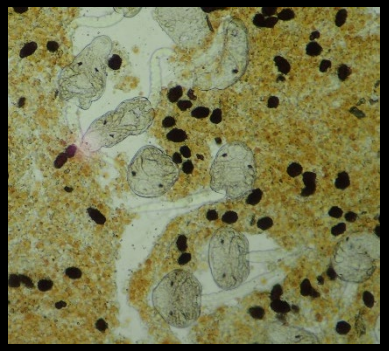
Selective and universal primers for trematode barcoding in freshwater snails

J. Routtu · D. Grunberg · R. Izhar · Y. Dagan · Y. Guttel · M. Ucko · F. Ben-Ami



Results: Parasite Identification

eyes, single tail, short

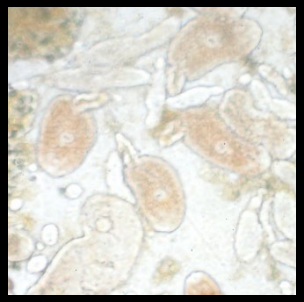


Heterophyidae: *Centrocestus formosanus*
Range Extension- Gill Fluke

eyes, single tail, long

Heterophyidae: *Haplorchis pumilio*
Range Expansion- Muscle Fluke

No eyes, single tail, short

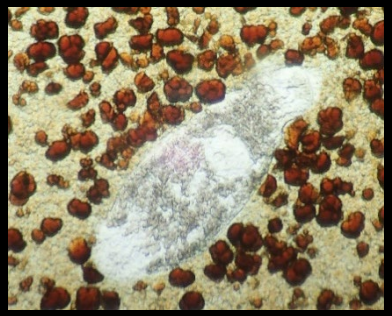


Renicolidae sp.
North American Record-
Kidney Fluke

no eyes, single tail, stylet

Lecithodendriidae sp.
North American Record-
bat Fluke

no eyes, peanut



Philophthalmidae:
Philophthalmus gralli
Range Extension- Eye Fluke

In summary

- *Melanoides tuberculata* in Florida do have trematode parasites.

- More surveys will likely yield more species.

Questions of surveys

- Do *M.tuberculata* populations in FL harbor trematode parasites? Yes
- Who are the parasites? Yes

- Do *P. maculata* populations in FL harbor trematode parasites?
- Who are the parasites?

Questions

- Do *M.tuberculata* populations in FL harbor trematode parasites?
- Who are the parasites?

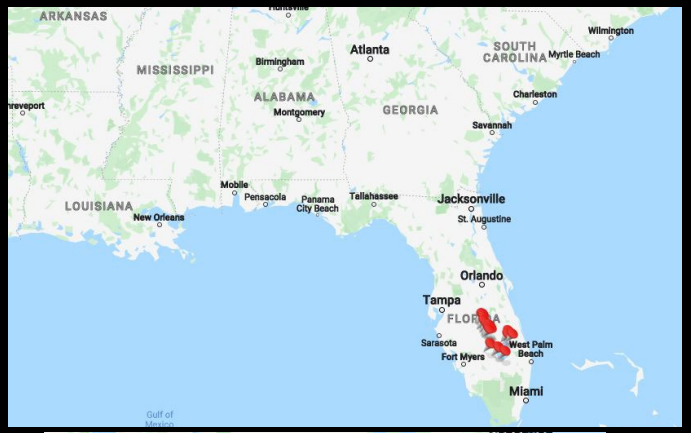
- Do *P. maculata* populations in FL harbor trematode parasites?
- Who are the parasites?

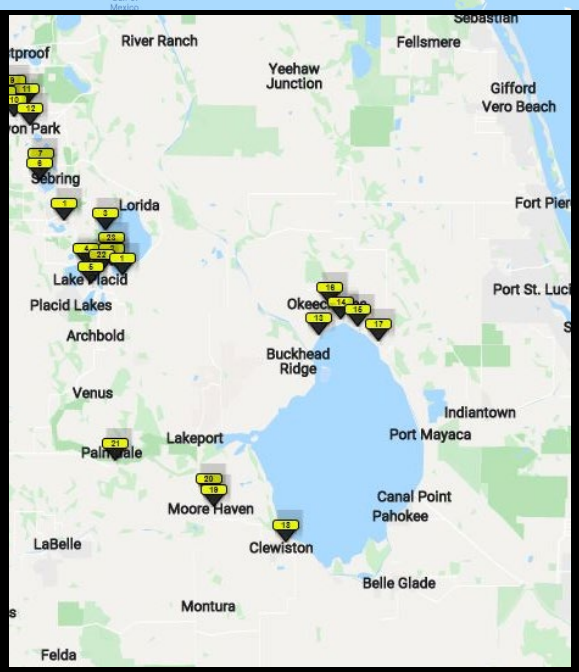
New Study Title

- “The best laid plans of mice and men most often go awry”…. Steinbeck

- Do *P. maculata* populations in FL harbor trematode parasites?
- Who are the parasites?

Methods: Finding the snails





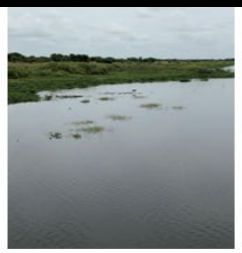
July and August 2021

25 sites, 90 miles

Methods: Finding the snails

Lake Istokpoga, site 2

Lake Istokpoga, site 3

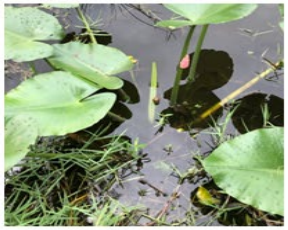


Lake Okeechobee, site 13

Un-named pond, Site 10

Taylor Creek, Site 16

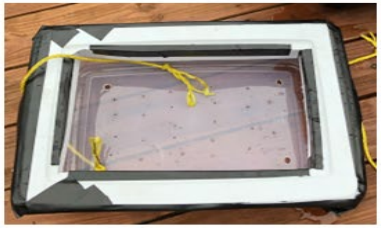
Clay Lake, Site 23



Giant Apple Snail eggs, site 3

Giant Apple Snail in trap, site 3

Isolated Apple Snails



Trap constructed from Rubbermaid bin

Van Dyke snail trap with alfalfa cube bait

Nearest City	Water Body	*P. maculata*						
		eggs	middens	male		female		
				#	N+	#	T+	
Lake Placid	Lake Istokpoga	N	N					
Lake Placid	Lake Istokpoga	Y	Y					
Lorida	Lake Istokpoga	Y	Y	5		2		
Lake Placid	Lake June in Winter	Y	Y	1	**1**	5		
Lake Placid	Lakes Pearl/Sirena	Y	Y					
Sebring	Lake Jackson	N	N					
Sebring	Lake Jackson	N	N					
Avon Park	Lake Olivia	N	N					
Avon Park	Lake Adelaide	N	N					
Avon Park	unnamed Pond	Y	Y	5	**1**	5		
Avon Park	Lake Lillian	N	N					
Avon Park	Lake Verona	Y	Y					
Taylor Creek	Lake Okeechobee	Y	Y	6	**1**	13		
Taylor Creek	Taylor Creek	Y	Y					
Okeechobee	Lake Okeechobee	Y	Y	1		0		
Okeechobee	Taylor Creek	Y	Y					
Okeechobee	Up the Grove Beach	Y	Y					
Clewiston	Okeechobee	Y	Y					
Moore Haven	Caloosahatchee Canal	Y	Y	2		3		
Moore Haven	Caloosahatchee Canal	Y	Y	1		0		
Palmdale	Fish Eating Creek	Y	Y					
Lake Placid	Lake Clay	Y	Y	14		18	**1**	
Lake Placid	Lake Istokpoga	Y	Y	1		0		
Sebring	Lake Josephine	Y	Y					

Methods- Parasite Detection

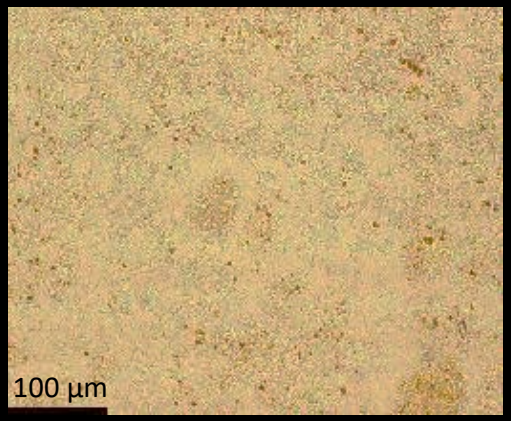
Snails cracked, tissues removed

Tissue smear, observed under compound microscope

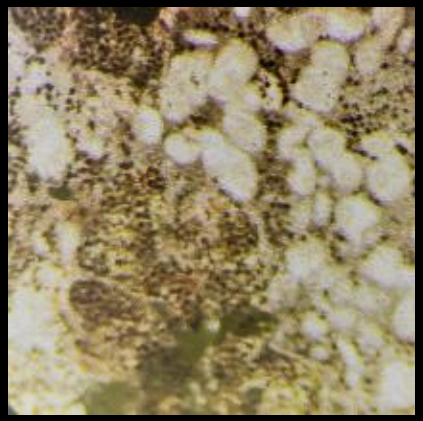
Snails brought to “lab” live, in stream water.

- Dissected ~ 80 snails

Methods: Finding the parasites



Nematode unknown
3 snails
3 sites



Trematode unknown
1 snail
1 site

Nearest City	Water Body	*P. maculata* eggs	middens	male #	male N+	female #	female T+
Lake Placid	Lake Istokpoga	N	N				
Lake Placid	Lake Istokpoga	Y	Y				
Lorida	Lake Istokpoga	Y	Y	5		2	
Lake Placid	Lake June in Winter	Y	Y	1	**1**	5	
Lake Placid	Lakes Pearl/Sirena	Y	Y				
Sebring	Lake Jackson	N	N				
Sebring	Lake Jackson	N	N				
Avon Park	Lake Olivia	N	N				
Avon Park	Lake Adelaide	N	N				
Avon Park	unnamed Pond	Y	Y	5	**1**	5	
Avon Park	Lake Lillian	N	N				
Avon Park	Lake Verona	Y	Y				
Taylor Creek	Lake Okeechobee	Y	Y	6	**1**	13	
Taylor Creek	Taylor Creek	Y	Y				
Okeechobee	Lake Okeechobee	Y	Y	1		0	
Okeechobee	Taylor Creek	Y	Y				
Okeechobee	Up the Grove Beach	Y	Y				
Clewiston	Okeechobee	Y	Y				
Moore Haven	Caloosahatchee Canal	Y	Y	2		3	
Moore Haven	Caloosahatchee Canal	Y	Y	1		0	
Palmdale	Fish Eating Creek	Y	Y				
Lake Placid	Lake Clay	Y	Y	14		18	**1**
Lake Placid	Lake Istokpoga	Y	Y	1		0	
Sebring	Lake Josephine	Y	Y				

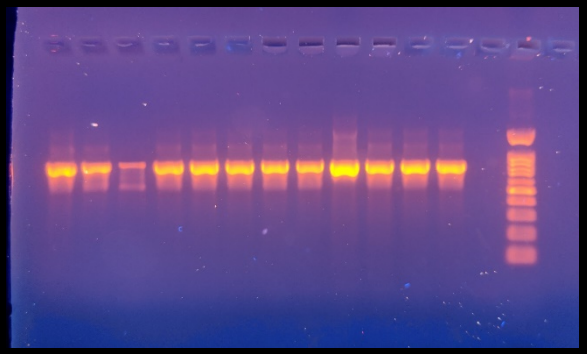
Parasite Identification….

DNA Extraction

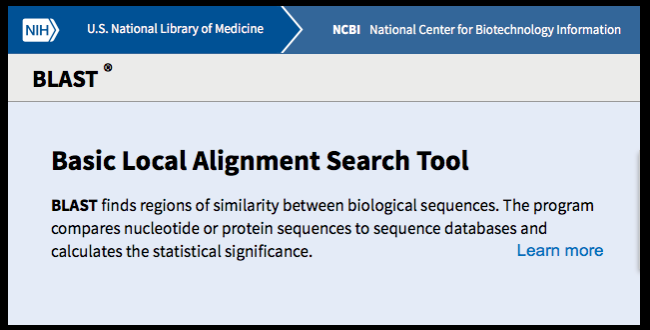
- Qaigen Powersoil Extraction Kit

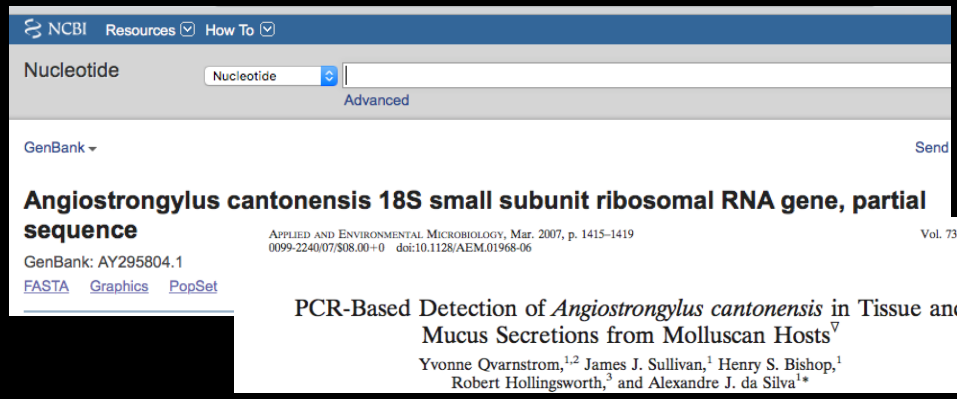
DNA amplification:

- General Nematode Primers (CO1)
- Targeted *A. cantonensis* primers (CO1)
- Trematode Barcoding Primer (18S)



Sequencing

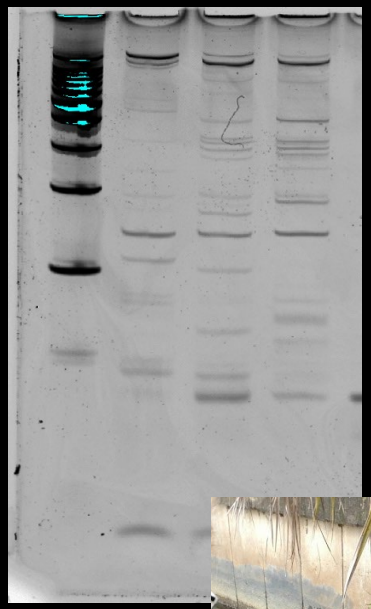




And we are still having trouble with the markers

Conclusions and “to dos”

- Molecular Markers are still good!
  - Sequencing help – JSU Geneticist
  - Positive Controls from CDC



- Detection Probability is too low
  - Solution: better bait for trapping

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GULF STATES MARINE FISHERIES COMMISSION

Acknowledgements