


"Innovation, Translation, and Impact in Tropical Medicine"

Environmental DNA: a different approach for food/water borne helminths studies

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Scenario of NTDs in Endemic areas

Indigenous cases acquired in their own community are observed among local people

- **Related to low sanitary standarts**
- **Related to low education / information**
- **Related with the environmental conditions**

Foodborne and waterborne diseases, as opisthorchiasis and schistosomiasis, are major threats to human health because their infective forms can be present in the daily life of everyone in endemic areas.

Usually the pathogen detection techniques requires sampling from hosts

- Fecal samples
- Serology is useful for diagnosis by detection of antibodies or antigens
- Other material other than serum samples can be used for detection of Ab or Ag
- Molecular techniques are applicable for diagnosis using any source of DNA

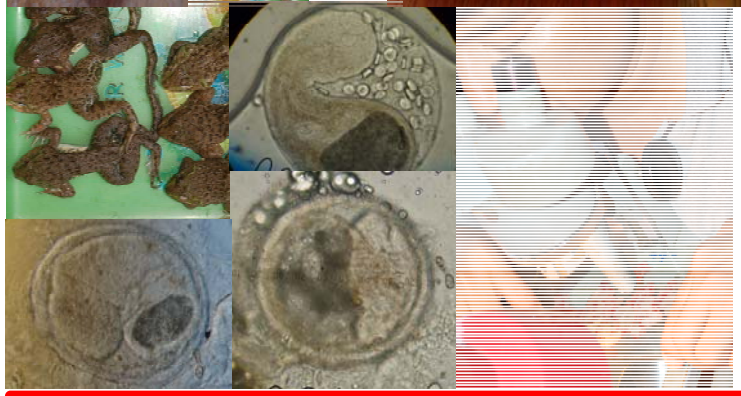
the most part of the time we are focusing in humans…

But… What is the whole situation?

What is the connection of “disease” in an ecosystem

To study these connections: Ecological Survey

Study the species related with the disease in a determined area: hosts, reservoirs, vectors, etc. understand their role in the **ecologic network of the pathogen** to describe the epidemiological scenario of the disease in the area.



Example opisthorchiasis:

- Cercariae shedding method (**Bithynia snail**)
  Prevalence of OV in snails less than 1%
- Metacercariae (MTC) examination (**cyprinid fish**)
  18/970 (1.9%) Ov MTC (+) Sato *et al.*, 2014

Limitations of the classical methods

- **Require high skilled techniques, manpower, time to perform, sometimes dangerous**
- **Difficult to track the distribution changes/apply in wide-ranging studies**
- **Bias in sampling can occur**

To overcome the limitations of classical methods as a new/complementary alternative in ecological surveys

Environmental DNA

Technique to study the biota from environmental samples (water or soil) by detecting species/genus specific DNA (Ficetola *et al.* 2008; Minamoto *et al.* 2012)

DNA found in the environment

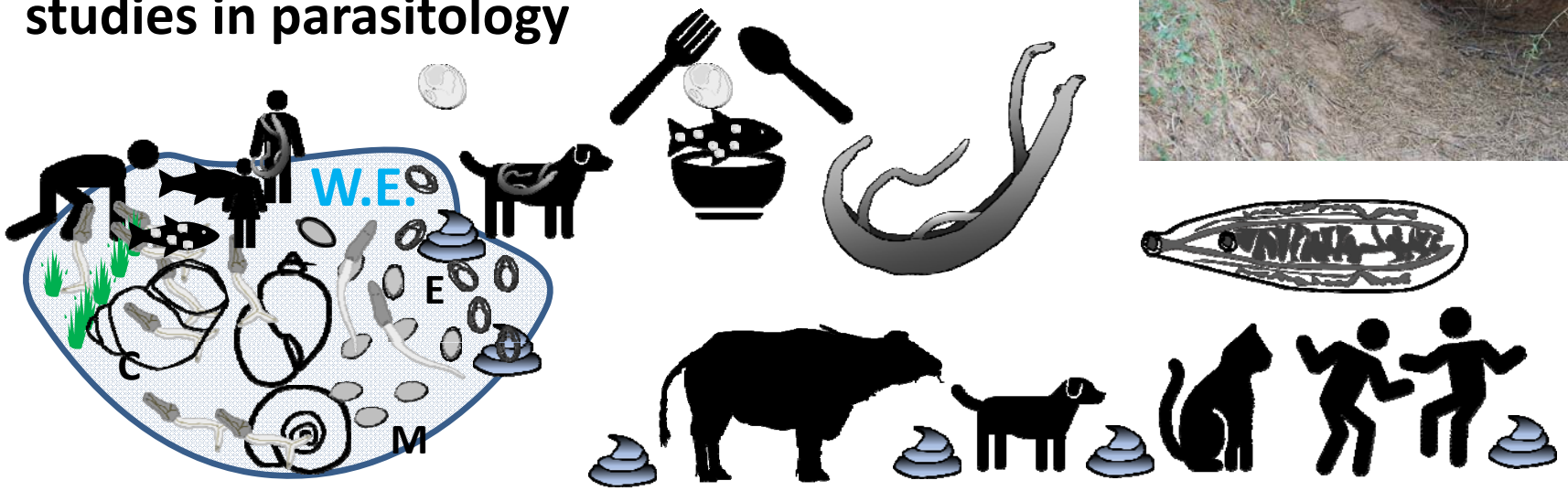
With several origins:

Excretion, secretion, exfoliation, reproduction, decomposition

and states:

intra/extra membranes, particulate and free

Now we are applying this technique for studies in parasitology



Methodology

qPCR

Specific primer/probe design

✓Short Length products: 100 to 200 bp desirable

✓DNA target: *CO1,* ITS (or other)

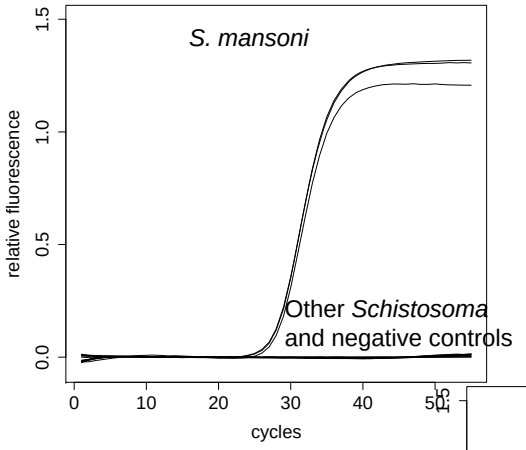
✓Specificity check in silico: database

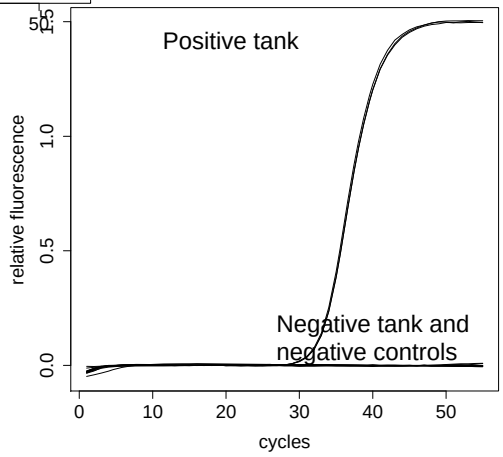
Determination of specificity/ sensitivity

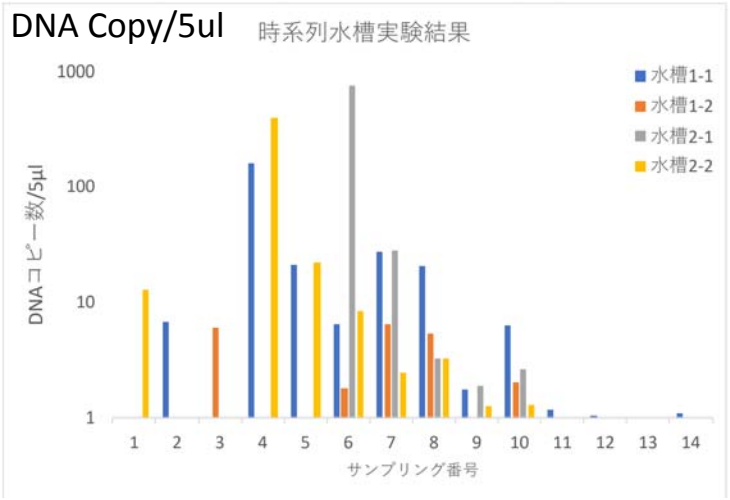
✓Compare with conventional PCR using known samples (from specimens and experimental infection water)

✓Test the system using non-target DNA

✓Confirm PCR positive samples by sequencing



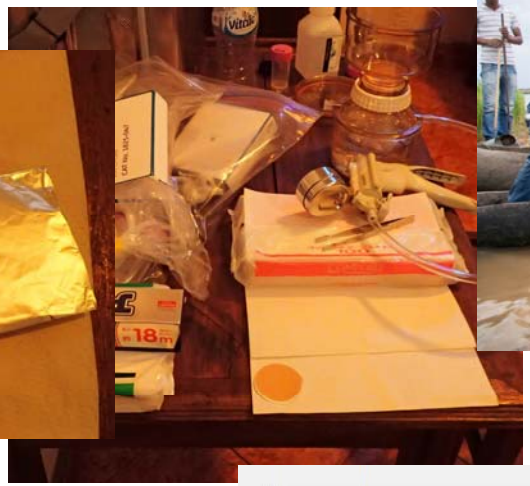




Methodology

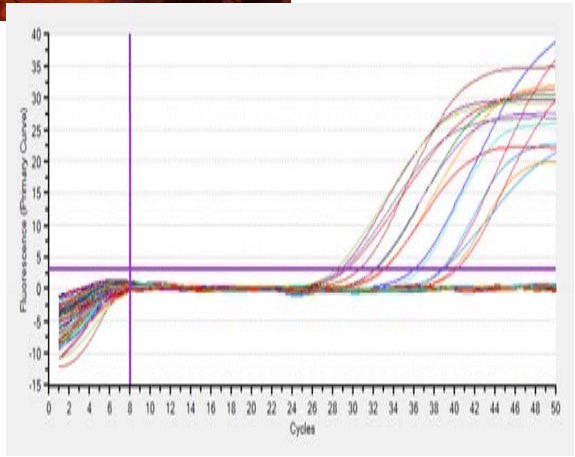
In the field

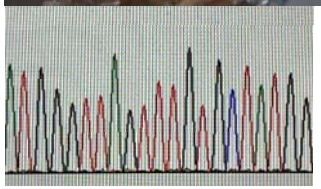
- Samples collection (500ml each)
- Filtration of water (0.7 µm pore glass fiber filter)
- Fixation in Ethanol



In the lab

- DNA extraction and purification
- PCR and qPCR
- Sequencing, NGS

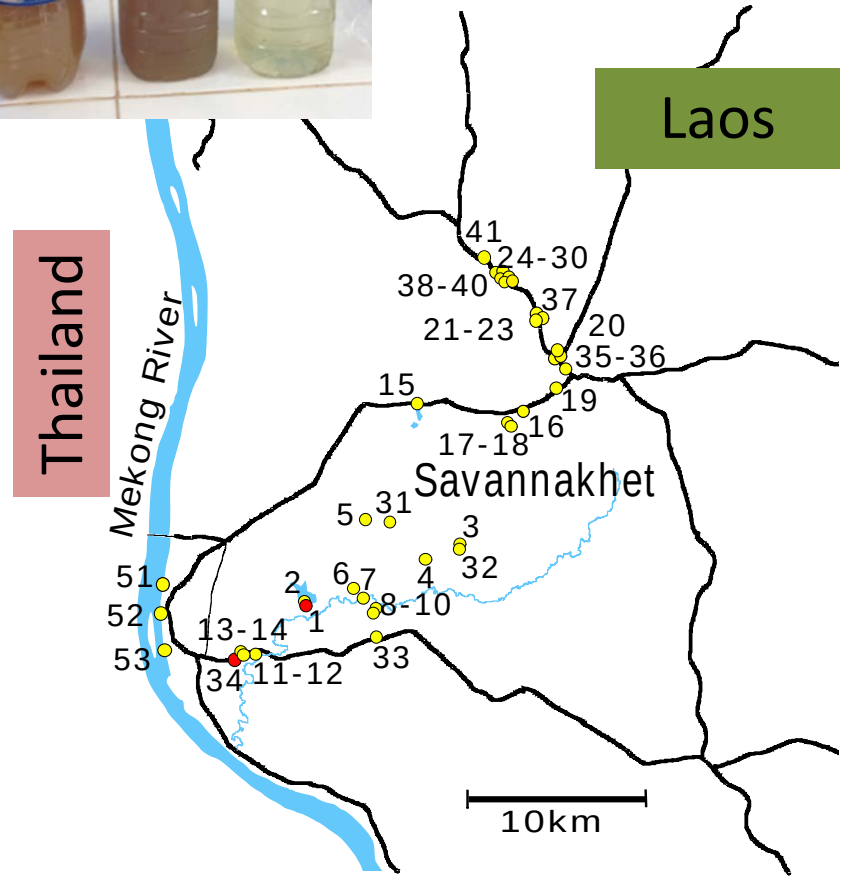




eDNA and opisthorchiasis in Lao-PDR

- Savhannakhet, Laos
- Total 44 points 62 samples

  Ponds, rivers, rice field etc.



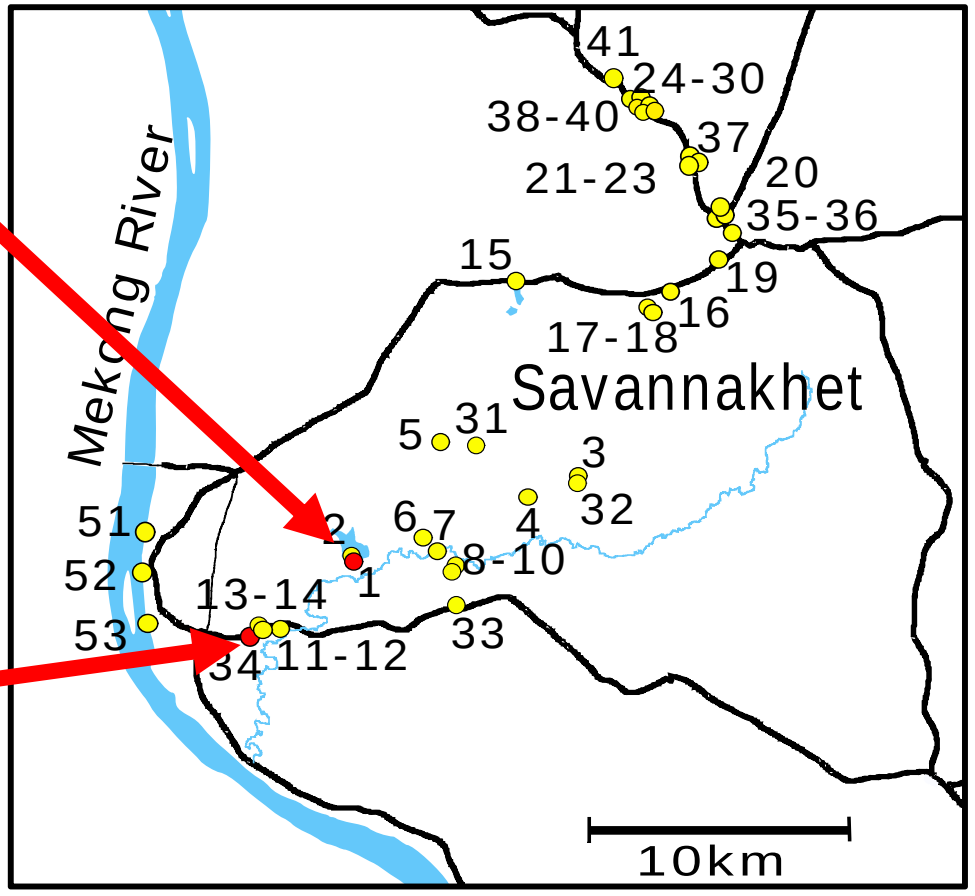
Hashizume et al., Acta Trop 169 (2017)

Results

Ov DNA was detected from 2 sites (pointed red)

Pond 1

Lotus flower pond



Detection of 1st intermediate hosts

- Detection of Bithynid Snail by PCR
- eDNA samples of environmental water
- Difficult primer design (database info)

Detection of 2nd intermediate hosts

- Same DNA samples from environmental water
- PCR assay using the universal primers for fishes
- Next-generation sequencing

- Total 35 fish species were identified
- Including 9 species as host of Ov

35 fish species detected from environmental water

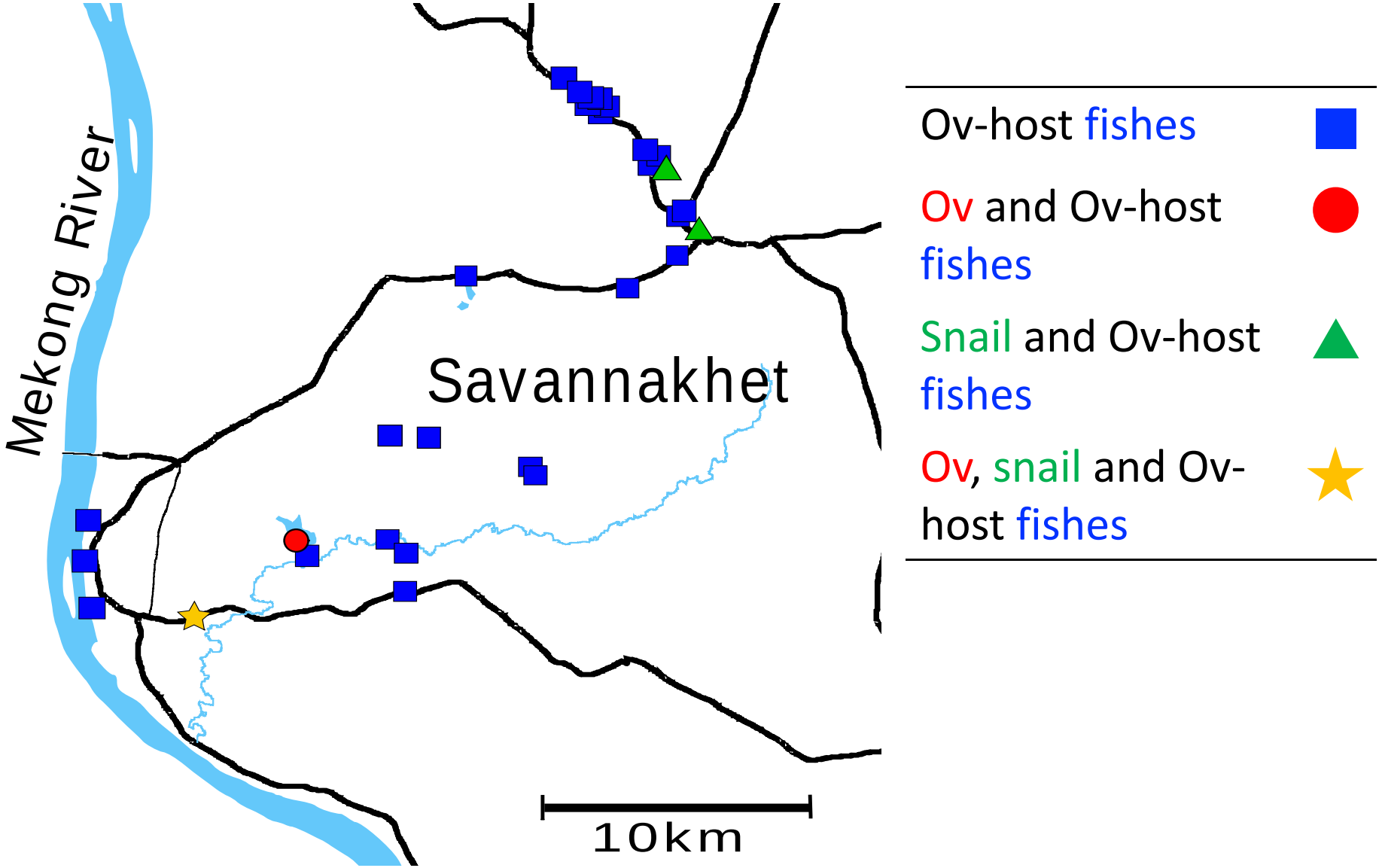
Scientific name	Family
●*Barbonymus gonionotus*	Cyprinidae
Catla catla	Cyprinidae
Cirrhinus cirrhosus	Cyprinidae
●*Cyclocheilichthys repasson*	Cyprinidae
Cyprinus carpio	Cyprinidae
●▲*Esomus metallicus*	Cyprinidae
Hampala dispar	Cyprinidae
Henicorhynchus lineatus	Cyprinidae
Hypophthalmichthys nobilis	Cyprinidae
Labiobarbus leptocheilus	Cyprinidae
Mystacoleucus marginatus	Cyprinidae
●*Osteochilus hasseltii*	Cyprinidae
Osteochilus salsburyi	Cyprinidae
Probarbus jullieni	Cyprinidae
▲*Puntius aurotaeniatus*	Cyprinidae
Raiamas guttatus	Cyprinidae
Acantopsis sp	Cobitidae
●*Clupeichthys aesarnensis*	Clupeidae

Scientific name	Family
Gambusia affinis	Poeciliidae
Clarias fuscus	Clariidae
Clarias gariepinus	Clariidae
Ailia sp	Schilbeidae
Pangasius larnaudii	Pangasius
Sperata seenghala	Bagridae
●▲*Anabas testudineus*	Anabantidae
Nandus nandus	Nandidae
Channa marulius	Channidae
Giuris margaritacea	Eleotridae
Oxyeleotris marmorata	Eleotridae
Oreochromis aureus	Cichlidae
Oreochromis niloticus	Cichlidae
▲*Oreochromis sp. 'red tilapia'*	Cichlidae
●▲*Trichopodus trichopterus*	Osphronemidae
Betta splendens	Osphronemidae
Macrognathus pancalus	Mastacembelidae

Ov-host fishes are written by red-letter,

● were the species detected in No.1 Pond, ▲ were detected in No.34 lotus pond

Epidemiological scenario for opisthorchiasis in Savannakhet



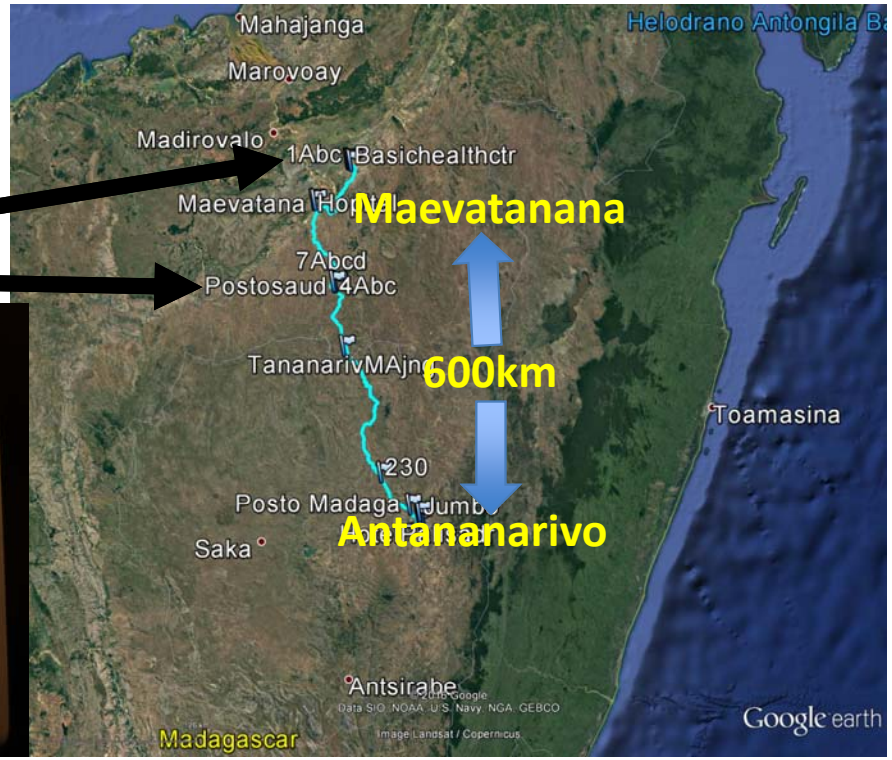
eDNA and Schistosomiasis in Madagascar

Local of study:
Maevatanana area
- mae = bad
- vatana = beautifull
- tanana = village

Local of bichuba = bigbelly

Schistosoma mansoni* and *S. haematobium

Clinical cases



Sato et al., Int J Inf Dis (2018)

Water sampling

- 7 spots (21 samples of 500ml)
- 3 in *S. haematobium* cases area
- 4 in *S. mansoni* cases area

S. haematobium endemic area

Water used for agriculture, washing clothes, bath and drink. pH8.5-8.8, 25-27^{o}C. **no snails found**

S. mansoni endemic area

Water used for washing clothes, bath and for drink. pH8.5-8.8, 19-20^{o}C. **snails were found**

All eDNA samples were subjected to a blind test.
From the tested material **one spot was positive for *S. mansoni*, the sample 5**

- The sample 5: Taken in small river where the people use to take bath drink, washing clothes, etc in a *S. mansoni* cases area.
- That was the place where we could confirm *Biomphalaria pfeifferi* snails
- The real time PCR result was confirmed by conventional PCR and direct sequencing.
- We could test successfully our primer-probe set in the field with satisfactory specificity.

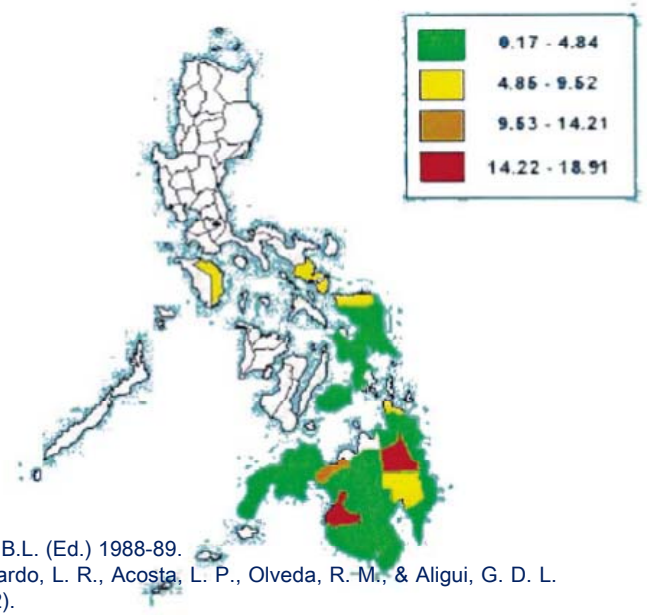


eDNA can be an useful tool for eco-epidemiology studies on schistosomiasis

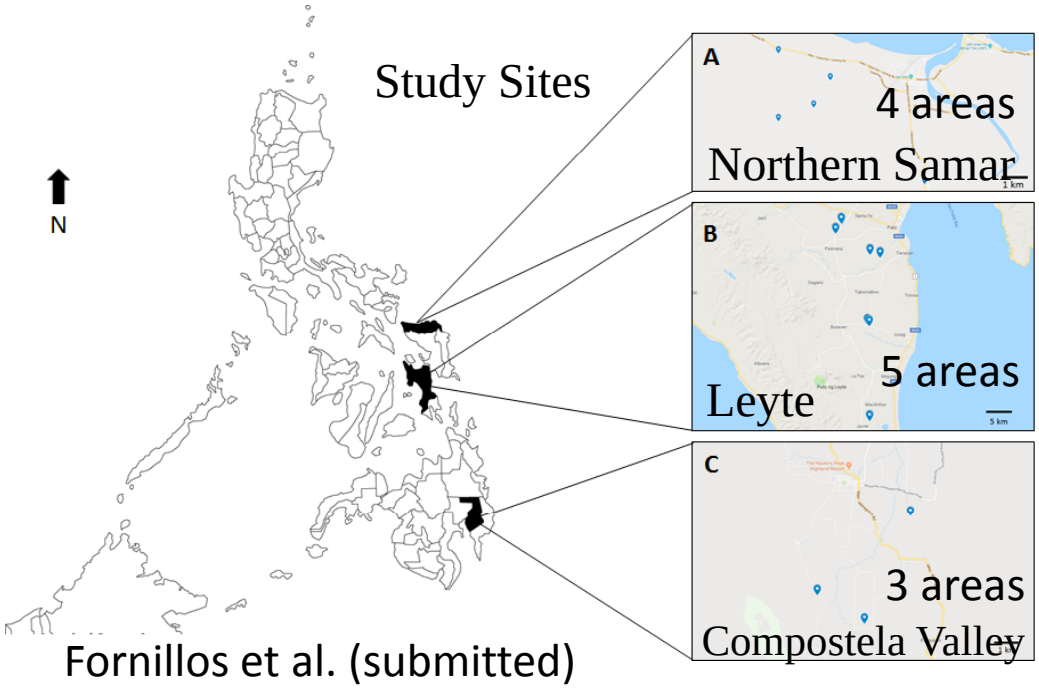
Development of an eDNA detection method for Philippine isolates of *Schistosoma japonicum* and *Oncomelania hupensis quadrasi* from field water samples

Schistosomiasis japonica in the Philippines

- Endemic in 28 provinces, 14 cities, 189 municipalities, and 2,221 barangays
- 12 million Filipinos are living in endemic areas and 2.5 million are directly exposed to infection
- New endemic foci discovered in 2002 and 2005 respectively in Gonzaga, Cagayan and 2005 in Calatrava, Negros Occidental



Blas, B.L. (Ed.) 1988-89.
Leonardo, L. R., Acosta, L. P., Olveda, R. M., & Aligui, G. D. L. (2002).
Leonardo et al. (2015).



Fornillos et al. (submitted)

Results: qPCR of Field Water Samples

Snails observed sites

+ Snail Site	Infection Rate	qPCR Results	
		S. japonicum	*O. h. quadrasi*
PINOPos	2.08	+	+
GDNSPos	0.43	+	+
CBRNPos	1.41	+	+
SOCSPos	0.24	-	+
CSLNPos	0.2	-	-
DITAPos	ND	+	+
TIG	ND	+	+
Total **7**	**5**	**5**	**6**

No snails sites (inspection)

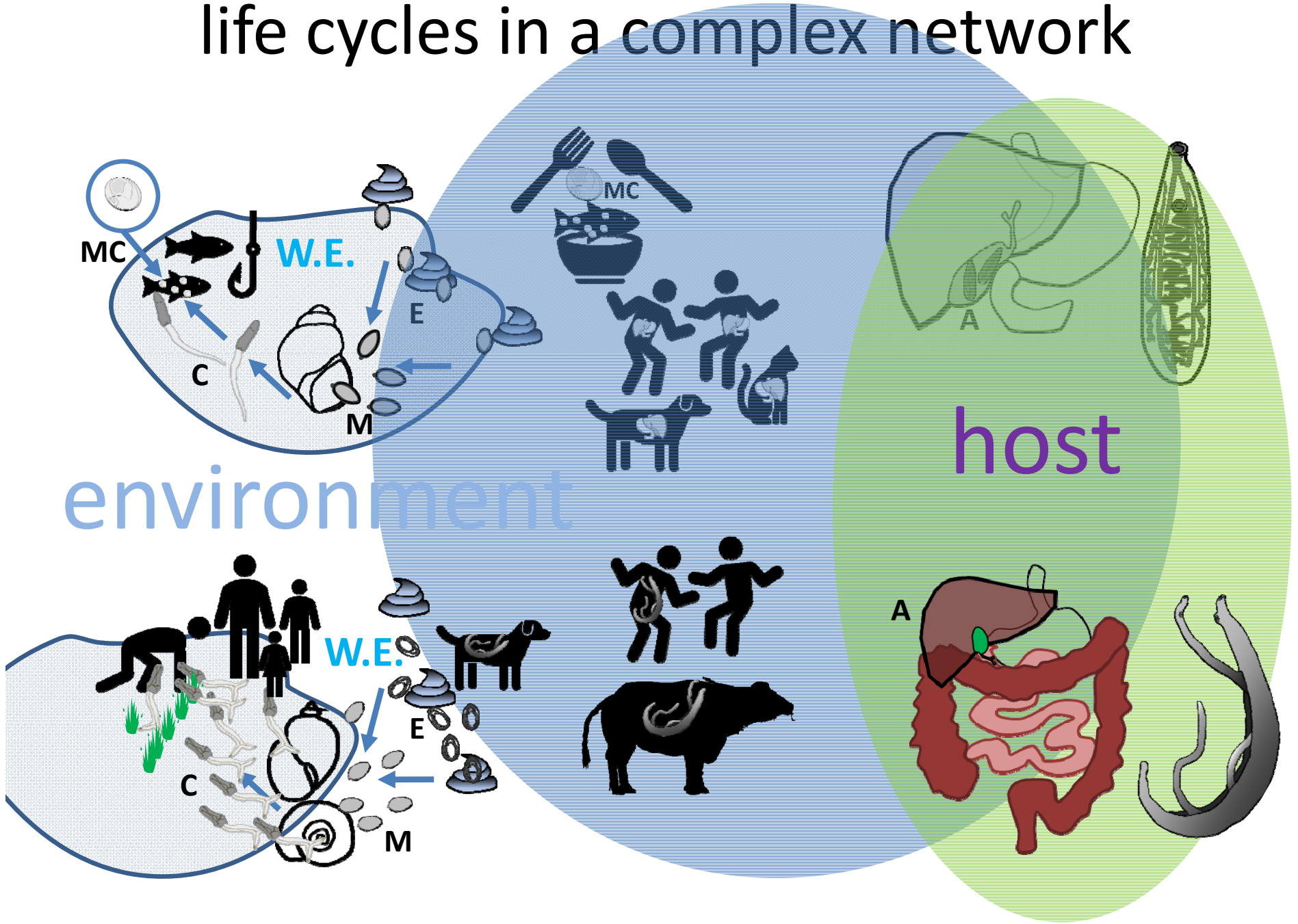
Snail Site	qPCR Results	
	S. japonicum	*O. h. quadrasi*
PINONeg	+	-
CBRNNeg	-	-
SOCSNeg	-	-
CSLNNeg	-	+
DITANeg	-	-
WAS	-	+
MGS	+	-
LIBW	-	-
LIBBH	-	-
OLR	-	+
MAP	+	-
NEP	-	-
Total **12**	**3**	**3**

***O. h. quadrasi* (Inspection Positive)**	
Snails detected	**7**
eDNA Positive	6
Inspection + eDNA	7
***S. japonicum* (*O. h. quadrasi* inspection positive)**	
Positive (microscopy)	5
eDNA Positive	5
Microscopy + eDNA	7

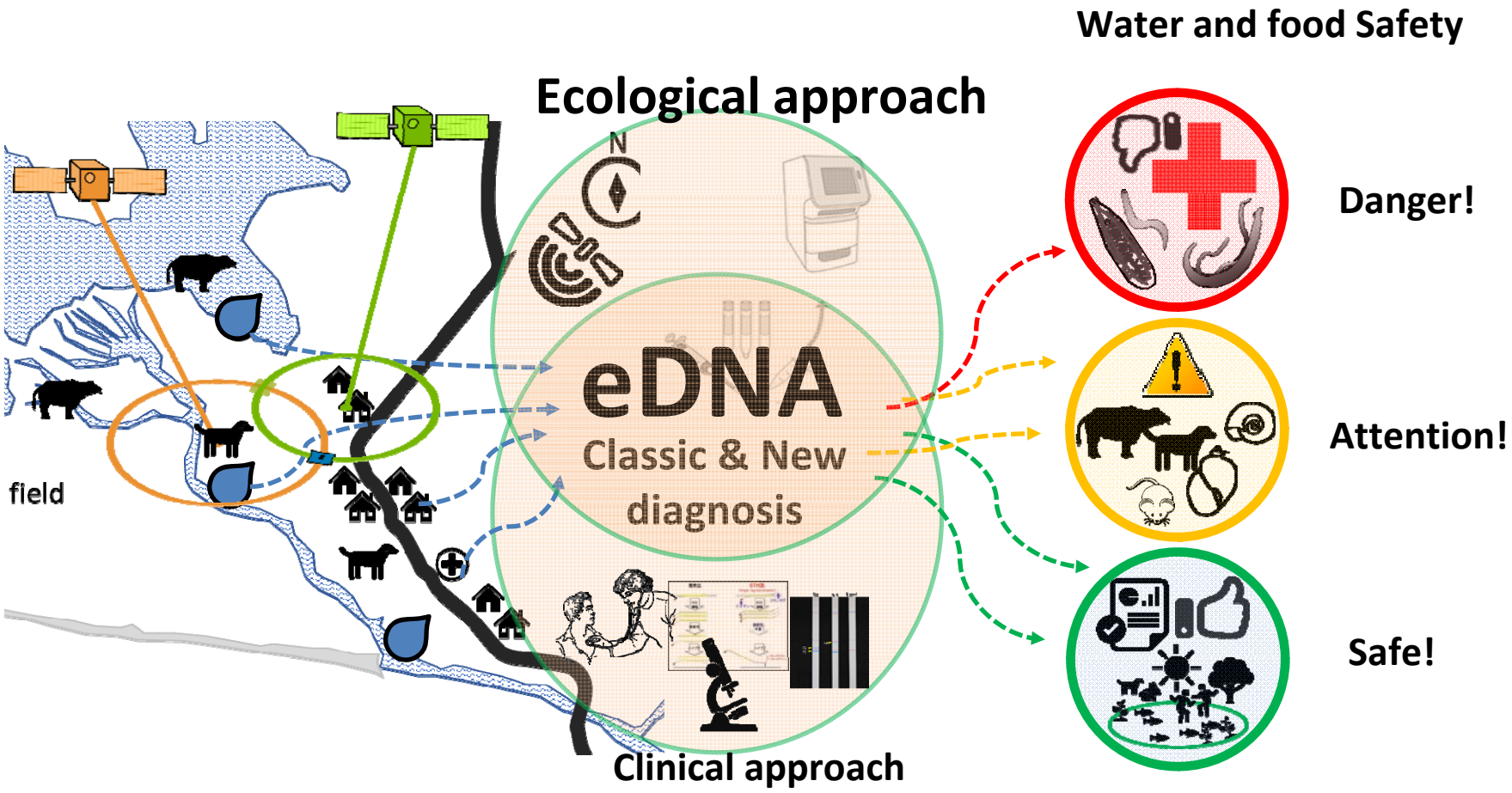
***O. h. quadrasi* (Inspection Negative)**	
No snail	12
eDNA Positive	**3**
***S. japonicum* (O.h. Negative)**	
eDNA Positive	**3**

Sj: Malacological surveys can be complemented with eDNA detection of both the snail and the parasite.

life cycles in a complex network



One-health



We hope to contribute with a more accurate surveillance technology to be applied in endemic areas, driving policies for the control of NTDs.

Thank you!