

# Morphological and molecular study of three populations of *Paragonimus mexicanus* Miyazaki e Ishii, 1968 (Digenea: Paragonimidae) in Mexico

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# Introduction

*Paragonimus mexicanus*

*Paragonimus* Braun, 1899

+/- 50 species in  
África, Asia & América

parasites in the lungs of mammals



# Introduction

*Paragonimus mexicanus*

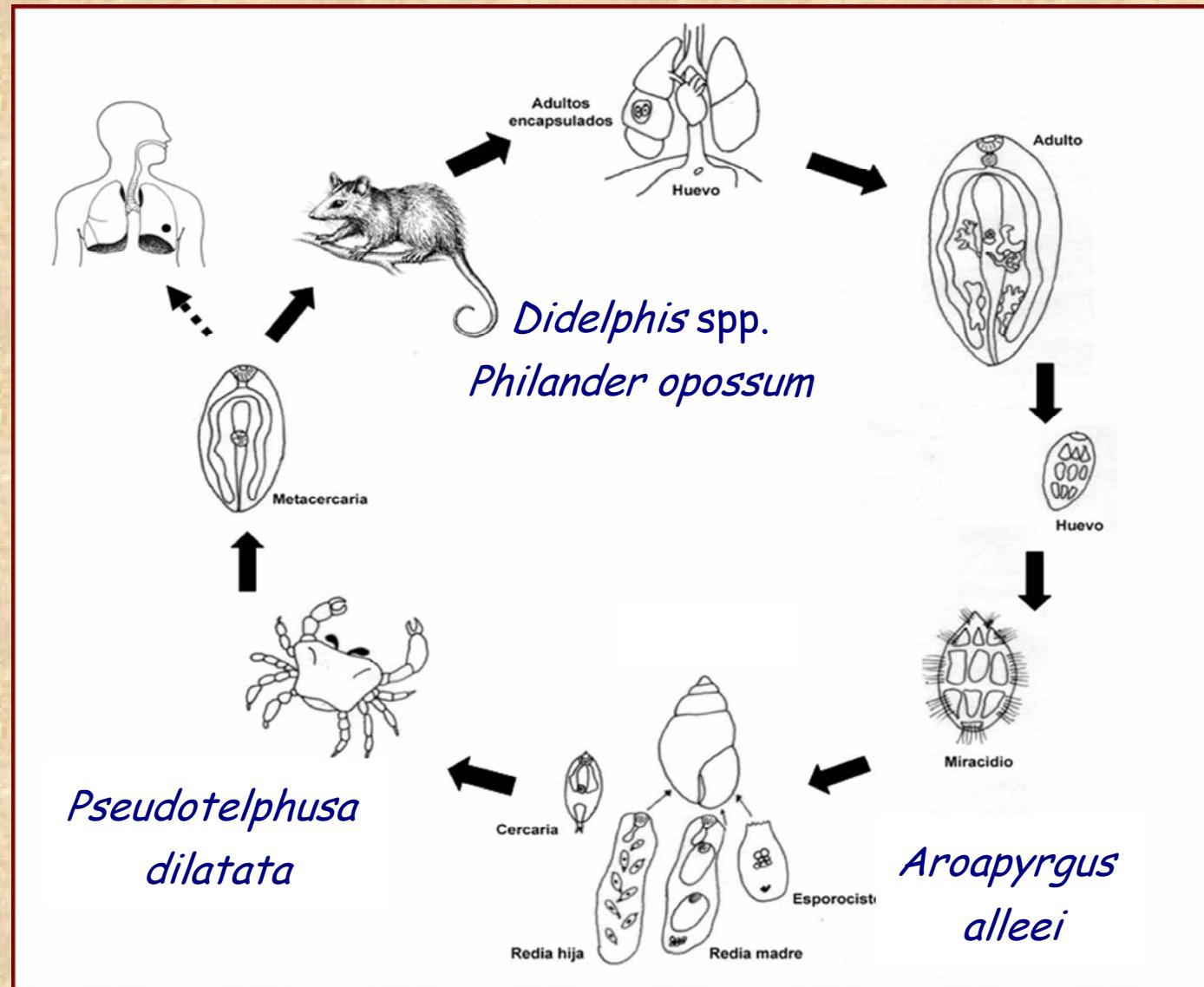
Nine (?) species in America

*Paragonimus mexicanus*

México  
Guatemala  
Costa Rica  
Colombia  
Ecuador  
Perú



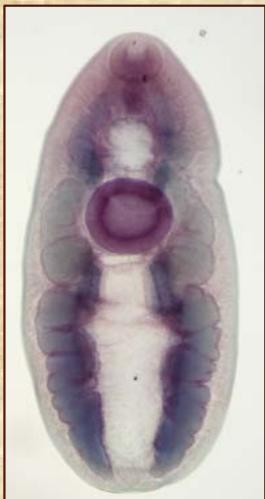
## Life cycle



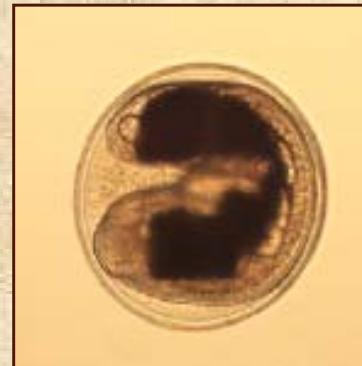
## Taxonomy of *Paragonimus*

### Metacercariae morphology

- cyst presence/absence
- cyst morphology



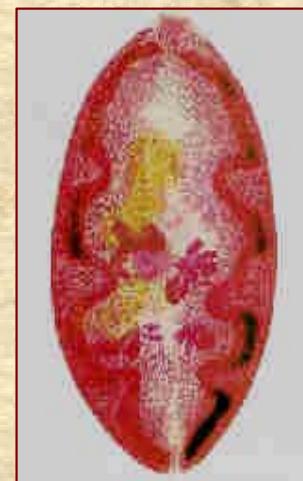
*P. mexicanus*



*P. harinatsutai*

### Adult morphology

- length/width ratio
- suckers diameter ratio
- acetabulum position
- shape and arrangement of spines in body surface
- size and lobulation of ovary and testes



*P. westermani*



*P. mexicanus*



*P. kellicotti*

# Introduction

[Jap. J. Parasit., Vol. 17, No. 5, 445-453 1968]

1968

*mexicanus*

Studies on the Mexican lung flukes, with special reference  
to a description of *Paragonimus mexicanus* sp. nov.  
(Trematoda: Troglotrematidae)

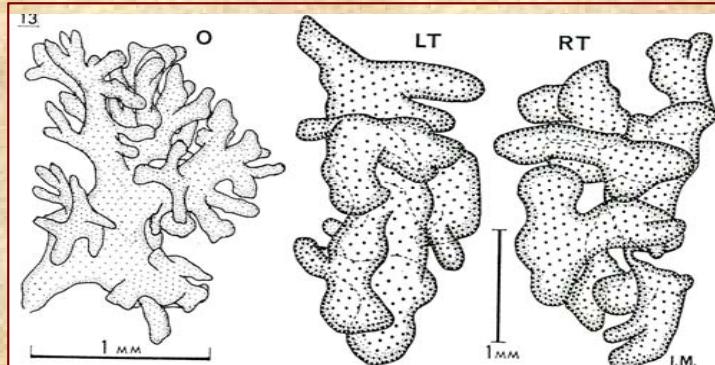
ICHIRO MIYAZAKI AND YOICHI ISHII

Department of Parasitology, Faculty of Medicine,  
Kyushu University, Fukuoka, Japan

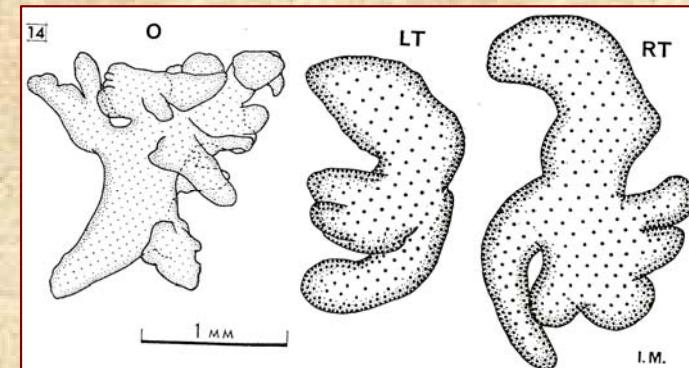
Ovary and testes ramification

Testes size

Suckers size



Species A  
*P. mexicanus* (n=26)



Species B  
*P. caliensis* (n=1)

# Introduction

Iwagami et al.

Journal of Parasitology (2000) 74, 315–322

2000

*Paragonimus mexicanus*

## Molecular phylogeographic studies on *Paragonimus westermani* in Asia

M. Iwagami<sup>1,7</sup>, L.Y. Ho<sup>2</sup>, K. Su<sup>3</sup>, P.F. Lai<sup>4</sup>, M. Fukushima<sup>1</sup>,  
M. Nakano<sup>1</sup>, D. Blair<sup>5</sup>, K. Kawashima<sup>6</sup> and T. Agatsuma<sup>7,\*</sup>

Wide distribution in Asia

Variable biology among populations

Partial sequences of ITS2 & COI

ITS2: 287bp [1pb] = 0.34 %

COI: (north-east / south) 383pb [44] = 11.5 %

Genetic differences congruent with  
biological differences

≠ species

# Introduction

Iwagami et al.

*Journal of Helminthology* (2003) 77, 33–38

2003

DOI: 10.1079/JOH2002147

*us mexicanus*

A molecular phylogeographic study based  
on DNA sequences from individual  
metacercariae of *Paragonimus mexicanus*  
from Guatemala and Ecuador

M. Iwagami<sup>1</sup>, C. Monroy<sup>2</sup>, M.A. Rosas<sup>2</sup>, M.R. Pinto<sup>3</sup>,  
A.G. Guevara<sup>4</sup>, J.C. Vieira<sup>4</sup>, Y. Agatsuma<sup>5</sup> and T. Agatsuma<sup>1\*</sup>

Partial sequences of ITS2 & COI

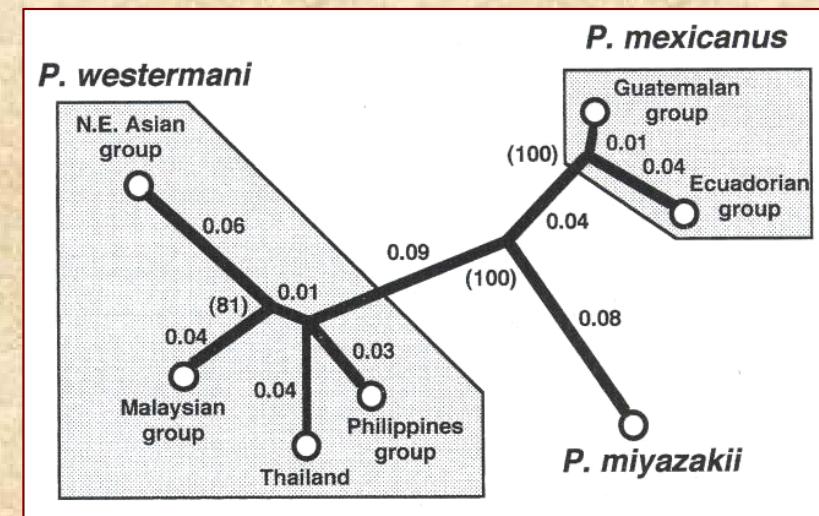
ITS2: 285 bp [1] = 0.35 %

COI: 383 bp [16] = 4.17 %

(< *P. westermani* in Asia)

*P. mexicanus* of Guatemala and  
Ecuador

↳ subspecies?



## general

- ↗ To determine if three populations of *Paragonimus* of Mexico correspond to *P. mexicanus* or to different taxa, based on morphological and molecular characters.

## particular

- ↗ To use nuclear and mitochondrial markers to compare populations.
- ↗ To correlate morphological characters of preserved specimens of *P. mexicanus* with the molecular information obtained in this study.

# Methods

*Paragonimus mexicanus*

Collecting sites

Colima

Veracruz

Chiapas

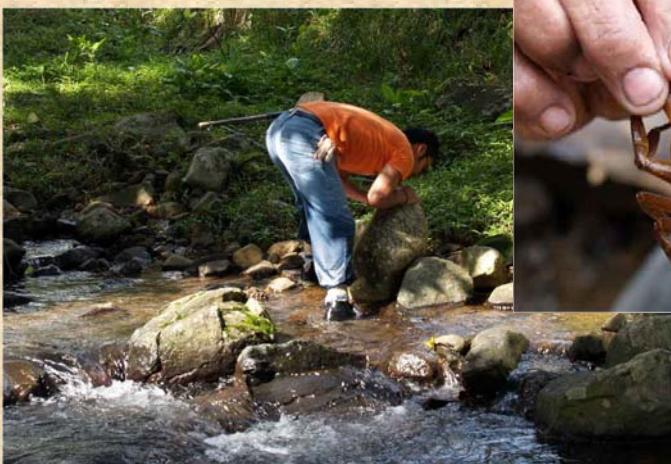
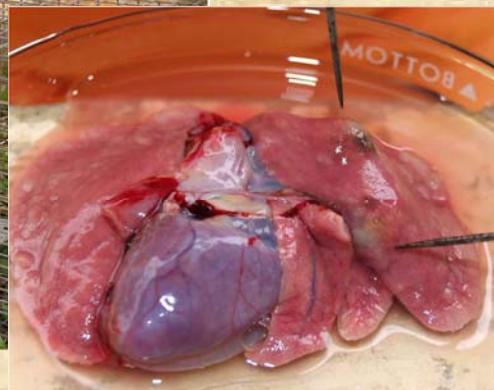
+ Guatemala & Ecuador  
Iwagami et al. 2003

*P. westermani*  
outgroup



# Methods

*Paragonimus mexicanus*



70% ethanol  
4% formalin

100% ethanol

Morphological study  
Light microscopy  
SEM  
Comparison with  
Collection specimens

Molecular study  
DNA extraction  
PCR  
Sequencing  
ITS2 & COxI  
Phylogenetic analysis

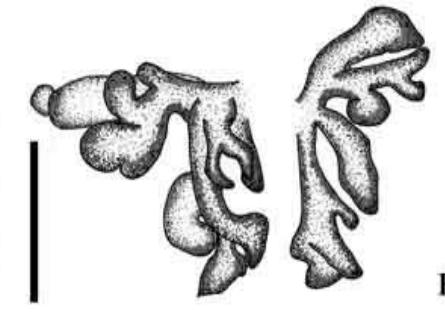
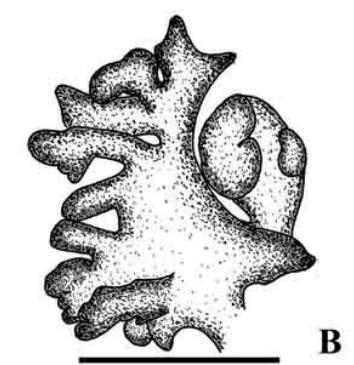
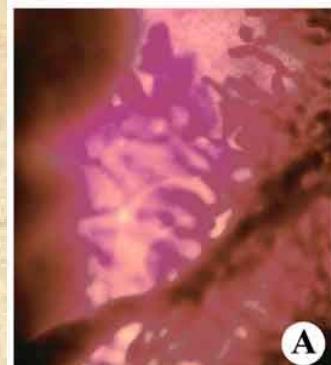
## Specimens obtained for this study

Locality	Host	Processing		Total
		Molecular	Morphology	
<b>ADULTS</b>				
Chiapas	<i>Didelphis virginiana</i>	6	5	
Colima	<i>Didelphis marsupialis</i>	5	5	
	<i>Didelphis virginiana</i>	5	5	
	<i>Philander opossum</i>	2	1	
	TOTAL ADULTS	18	16	34
<b>METACERCARIAE</b>				
Chiapas	<i>Raddaus tuberculatus*</i>	5	3	
Colima	<i>Pseudothelphusa aff. seiferti</i>	4	1	
Veracruz	<i>Tehuana poglayenorum*</i>	8	3	
	TOTAL M.C.	17	7	24

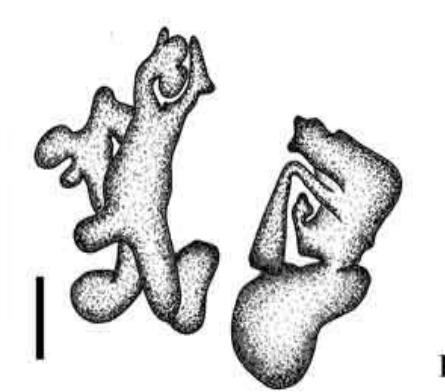
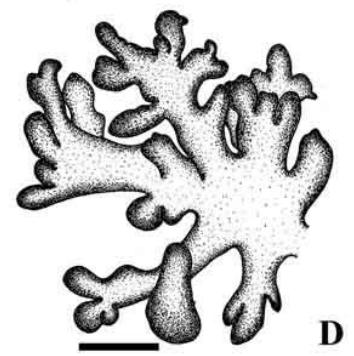
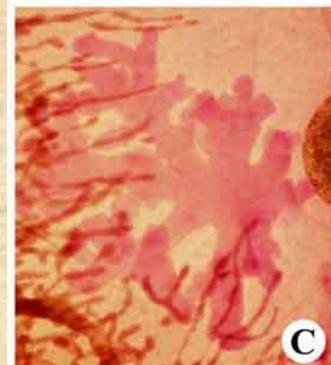
# Results: morphology of adults

*Paragonimus mexicanus*

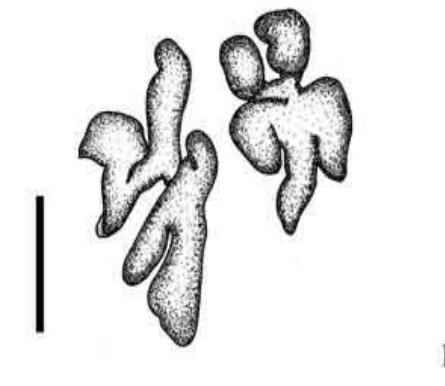
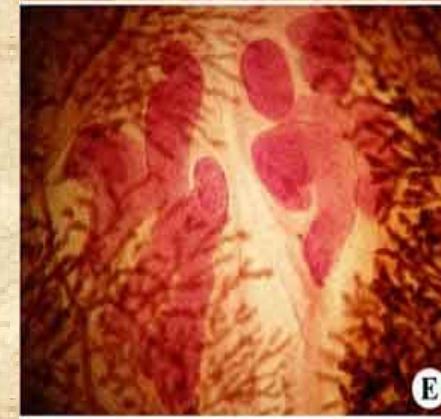
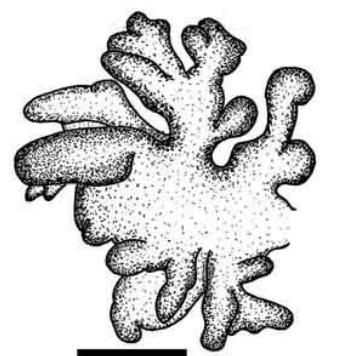
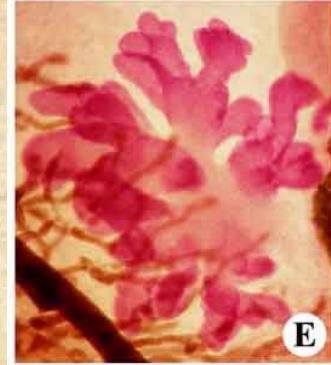
Chiapas



Colima



Veracruz



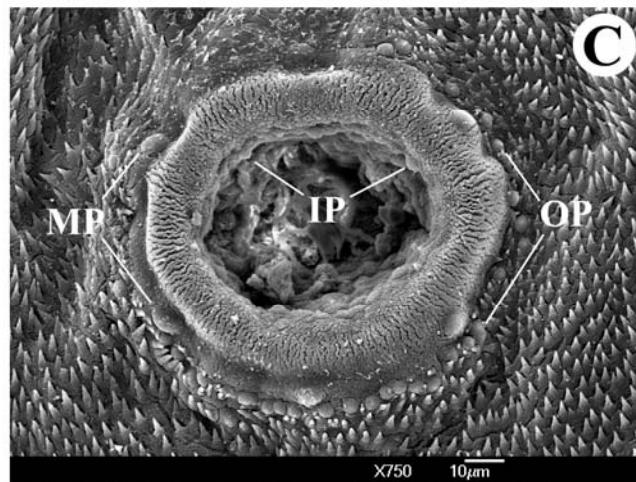
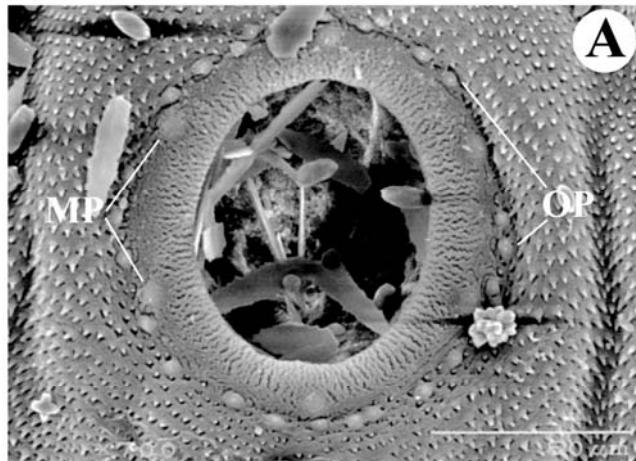
Ovario

Testículos

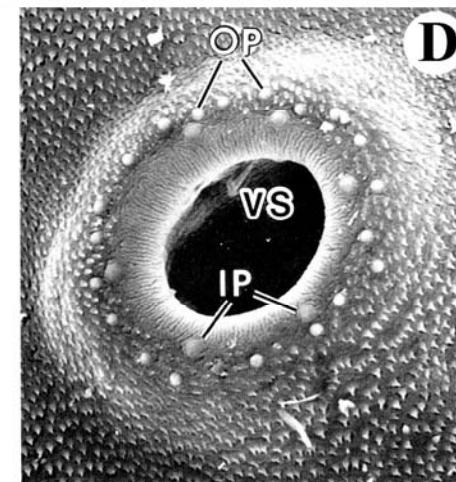
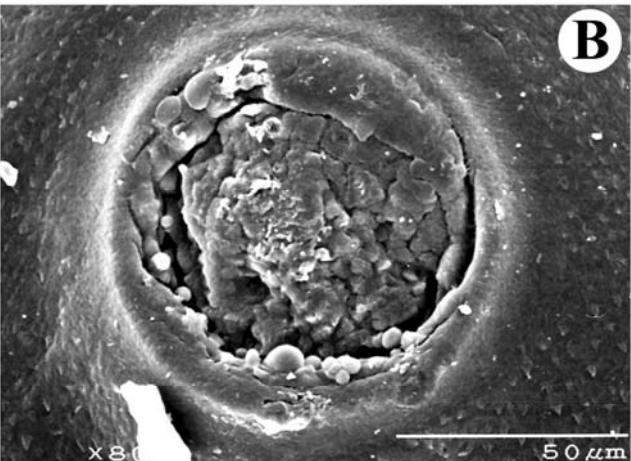
# Results: papillar pattern in m.c.

*Paragonimus mexicanus*

Chiapas  
6 int., 22 ext.



Veracruz  
6 int., 6 mid.  
22 - 31 ext.



Guatemala  
Tongu et al. (1995)  
6 int., 20 - 30 ext.

Colima  
no papillae

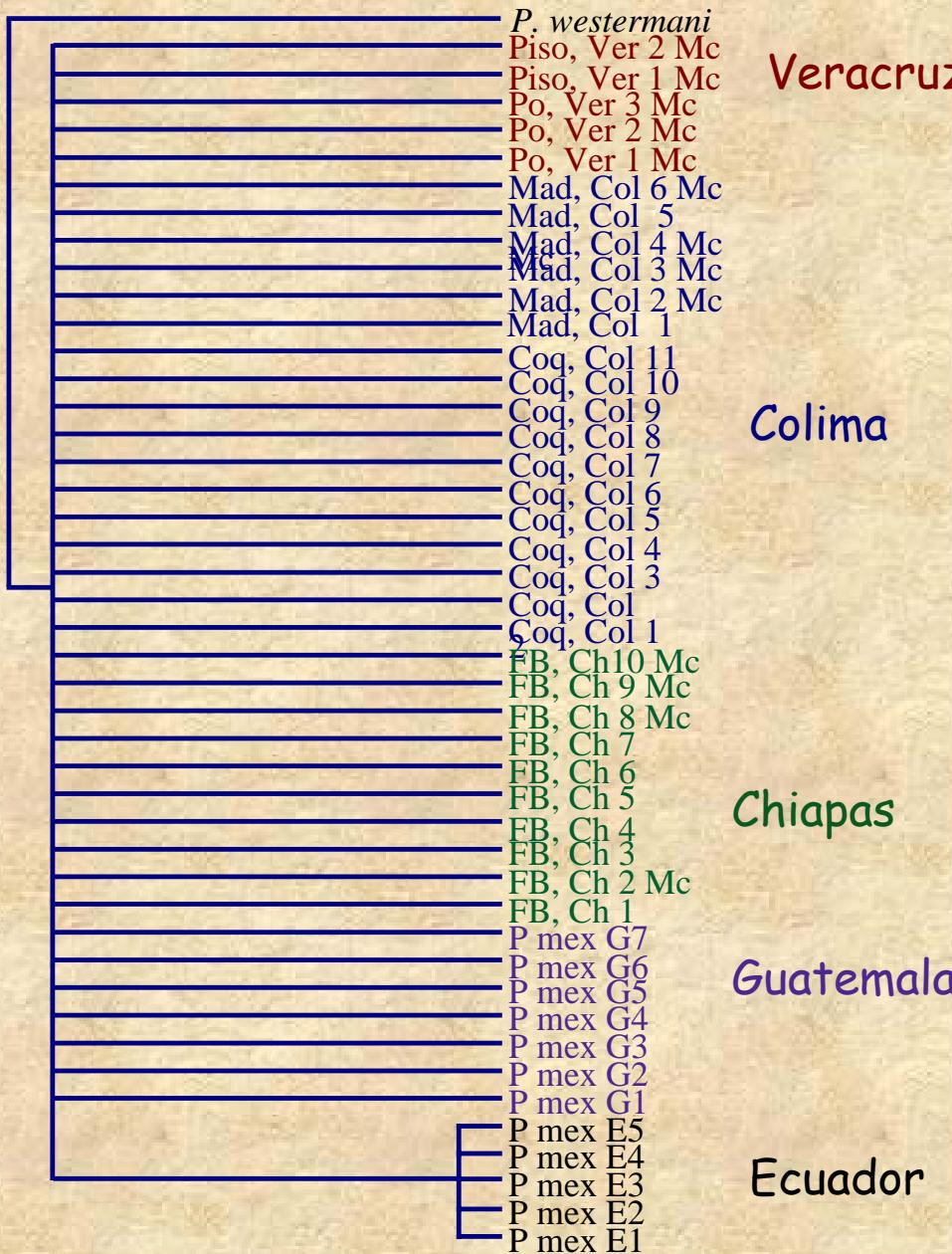
# Results: molecular divergence

*Paragonimus mexicanus*

	ITS2		COI	
	# changes	%	# changes	%
outgroup vs ingroup	29-31	10.1-10.5	40-82	10.6-21.4
among ingroup populations				
Chiapas vs Colima	2-7	0.4-1.5	23-28	6.2-7.5
Chiapas vs Guatemala	1-4	0.2-0.9	0-5	0-1.3
Chiapas vs Ecuador	1-4	0.2-0.9	18-21	4.7-5.5
Chiapas vs Veracruz	0-3	0.0-0.7	16-18	4.4-4.9
Colima vs Guatemala	2-7	0.4-1.5	23-39	6.0-10.2
Colima vs Ecuador	3-8	0.7-1.8	26-39	6.8-8.36
<i>P. mexicanus</i>				
Iwagami et al. (2003)	1	0.35	18-21	4.6-5.5
Guatemala vs Ecuador				
<i>P. westermani</i>				
Iwagami et al. (2000)	7-11	2.46-3.87	24-39	6.3-10.2
NEAsia vs South Asia				
<i>Oligogonotylus</i> spp.				9.5-10.3
Razo-Mendivil et al. (2004)				
<i>Haematoloechus</i> spp.				6.1-12.2
León-Règagnon et al. (2005)				
<i>Glypthelmins</i> spp.				15.9-18.5
Razo-Mendivil et al. (2008)				

# Results: Phylogenetic analysis

*Paragonimus mexicanus*



ITS2

final alignment 285 sites  
5 informative characters

Strict consensus of 6 MPT  
heuristic search  
(100 replicates)

# Results: Phylogenetic analysis

*Paragonimus mexicanus*

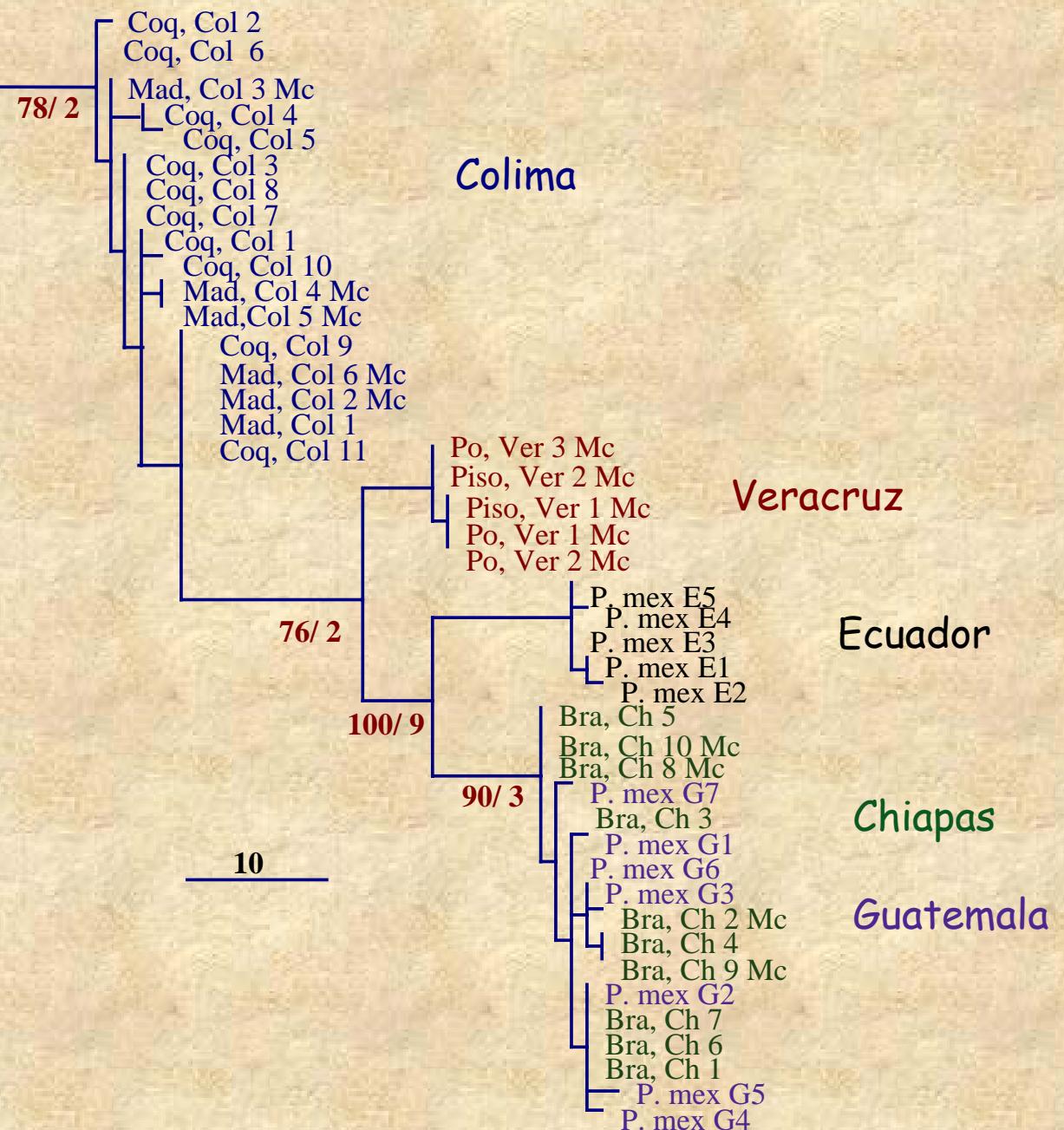
*P. westermani*

COI

final alignment 383 sites

49 informative characters

Strict consensus of 2 MPT  
heuristic search  
(100 replicates)



# Results: Phylogenetic analysis

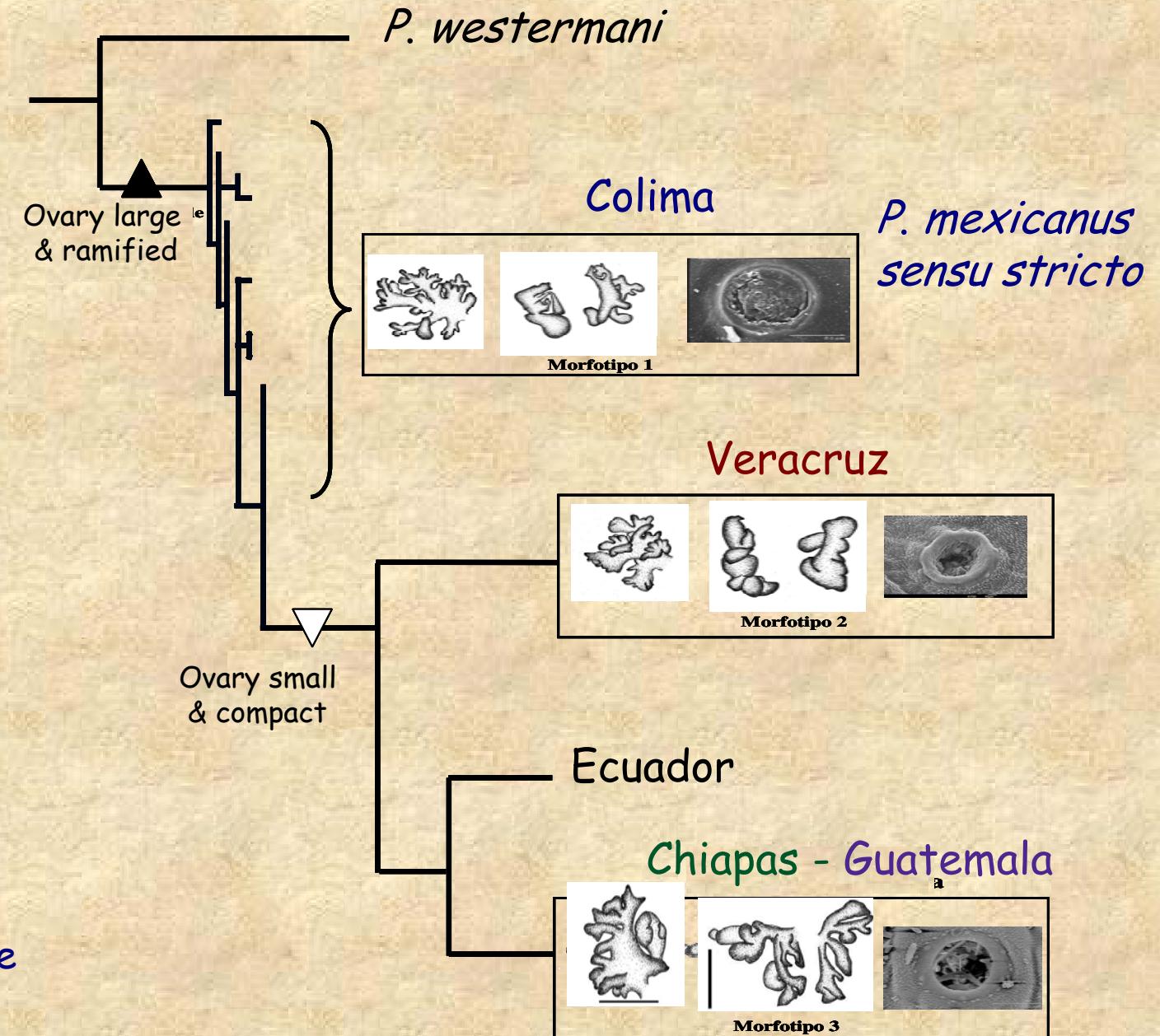
*Paragonimus mexicanus*

Genetic divergence  
Morphological variation  
Geographical distribution

congruent

Different species?

Additional marker  
Additional specimens  
Wider geographical range



# Acknowledgements

*Paragonimus mexicanus*

Special thanks to

Blanca Hernández for advice in project design

Rosario Mata, Alejandro Oceguera, Ricardo Paredes and  
Rosamond Coates for help in field collections

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