

FIVE YEAR REPORT

Department of Zoology, University of Jammu-180006

Project title: Human pulmonary paragonimiasis in crab eating communities and smear negative suspected TB cases from some states of India.

PI- Prof. Seema Langer

Co-PI- Dr. Rakesh Panjaliya

Date of Start: 15/10/2018

End of Project: 15/10/2023

Duration: Five years

Objective of the proposal: *Paragonimus metacercariae* recovery and crab Identification from various states of India.

Deviation made from original objectives if any, while implementing the project and reasons thereof: The survey stations were extended to Himachal Pradesh and Uttarakhand along with J&K for recovery of the parasite. This was done to ensure the recovery of the parasite.

Field/Experimental work giving full details of summary of methods adopted:

Study type

Active surveys: Cross-sectional door-to-door surveys were carried out in the selected villages for the detection of pulmonary paragonimiasis in selected fresh-water crab-eating communities.

Passive surveys: Suspected TB cases attending PHC/Microscopy centers in the selected villages were screened for pulmonary paragonimiasis.

Faunal surveys: Faunal surveys of freshwater crabs in selected study villages were performed and a parasitological examination of freshwater crabs was carried out.

Gene Sequencing: Molecular confirmation of freshwater crab species was based on sequencing of two mitochondrial genes (16S rRNA gene and cox1 gene) and two nuclear genes (28S rRNA and Histone3).

Results:

Active Survey:

Table: Result of IgG ELISA test on serum samples collected during active survey in the study state J&K (year wise data)																			
1st year				2 nd year				3 rd year				4th Year				5th Year			
Individuals Enrolled	Samples collected	Samples tested	Positive samples	Individuals Enrolled	Samples collected	Samples tested	Positive samples	Individuals Enrolled	Samples collected	Samples tested	Positive samples	Individuals Enrolled	Samples collected	Samples tested	Positive samples	Individuals Enrolled	Samples collected	Samples tested	Positive samples
2,790	4	4	0	172	0	0	0	1,810	17	17	0	901	223	223	0	1,842	254	254	0

A total of 7,515 people were enrolled for active survey of the various study stations for a total period of 5 years. Blood samples were collected from a total of 498 eligible subjects. All the collected blood samples were screened for antibodies against adult worm ES antigen of *Paragonimus* species by IgG ELISA.

Passive Survey:

Table: Result of IgG ELISA test on serum samples collected during passive survey in the study state J&K (year wise data)																
1 st year			2 nd year			3 rd year			4th Year			5th Year				
Samples collected	Samples tested	Positive samples	Samples collected	Samples tested	Positive samples	Samples collected	Samples tested	Positive samples	Samples collected	Samples tested	Positive samples	Samples collected	Samples tested	Positive samples		
7	7	0	9	9	0	28	28	0	339	281	0	186	244	0		

During the passive surveys, blood sample were collected from 569 TB-suspected patients attending PHCs/CHCs of various districts of Jammu & Kashmir. All the collected blood samples were screened for antibodies against adult worm ES antigen of *Paragonimus* species by IgG ELISA.

Crabs collection and morphological identification

5,649 freshwater crabs were collected in the villages from districts of Jammu & Kashmir, Himachal Pradesh, Uttarakhand. The crabs were identified as *Maydelliathelphusa masoniana*, *Sartoriana spinigera*, *Himalayapotamon jammuense*, *Acanthopotamon martensi*, *Himalayapotamon emphysetum*, *Himalayapotamon robertsonium*, *Himalayapotamon* sp., *Maydelliathelphusa harpax*, *Himalayapotamon garhwalense*, *Himalayapotamon kooloense*, *Hiamalayapotamon chambaensis*.

Table : Morphological identification and microscopy examination of crabs collected from the study area.

Total no. of crabs collected	Crab species identified	Locality
5,649	<i>Maydelliathelphusa masoniana</i>	Gho-manhan, Chadwal, Jammu district.
	<i>Sartoriana spinigera</i>	Chakrali village, Garhana wetland, Jammu district
	<i>Himalayapotamon jammuense</i>	Mangnar village, Poonch district
	<i>Acanthopotamon martensi</i>	Sai kalam village, Jammu district
	<i>Himalayapotamon emphysetum</i>	Jhajjar village, Jammu district
	<i>Himalayapotamon robertsianum</i>	Klai village, Poonch district
	<i>Himalayapotamon chambaensis</i>	Chambal, Himachal Pradesh
	<i>Himalayapotamon koolooense</i>	Rock Garden, HP
	<i>Himalayapotamon garhwalense</i>	Srinagar, Uttarakhand
	<i>Himalayapotamon koolooense</i>	Srinagar, Uttarakhand
	<i>Himalayapotamon emphysetum</i>	Bijrani zone, Jim Corbett NP, Uttarakhand
	<i>Maydelliathelphusa harpax</i>	Jim Corbett NP, Uttarakhand
	<i>Acanthopotamon martensi</i>	Jim Corbett NP, Uttarakhand



Maydelliathelphusa masoniana



Sartoriana spinigera



Himalayapotamon emphysetum



Himalayapotamon robertsianum



Himalayapotamon chambaensis



Himalayapotamon kooloense



Himalayapotamon sp.



Acanthopotamon martensi



Maydelliathelphusa harpax



Himalayapotamon garhwalense

Three species of crab were identified i.e. *Himalayapotamon emphysetum*, *Acanthopotamon martensi* and *Sartoriana spinigera* from Jim Corbett National Park, Uttarakhand. Sequence of two mitochondrial genes COX1 and 16srRNA of *Himalayapotamon emphysetum* which was collected from Uttarakhand has been generated and will be the first submission of the same in gene bank from the reserve area. A new species of freshwater crab *Himalayapotamon jammuense* has been identified from Poonch district of J&K, the description of which is still in progress. Further three new species belonging to genus *Himalayapotamon* sp. has been identified from Union Territory of Jammu and Kashmir.

Phylogenetic analysis: Molecular characterization of most of the identified crabs was carried out along with the phylogenetic analysis of the same. For the first time collection of crab was done from Jim Corbett National Park. Phylogenetic analysis of freshwater crabs from Jammu and Kashmir and Uttarakhand area was carried out. Three new species of crab from Jammu has been found. Sanger sequencing of other already known freshwater crab species has been done.

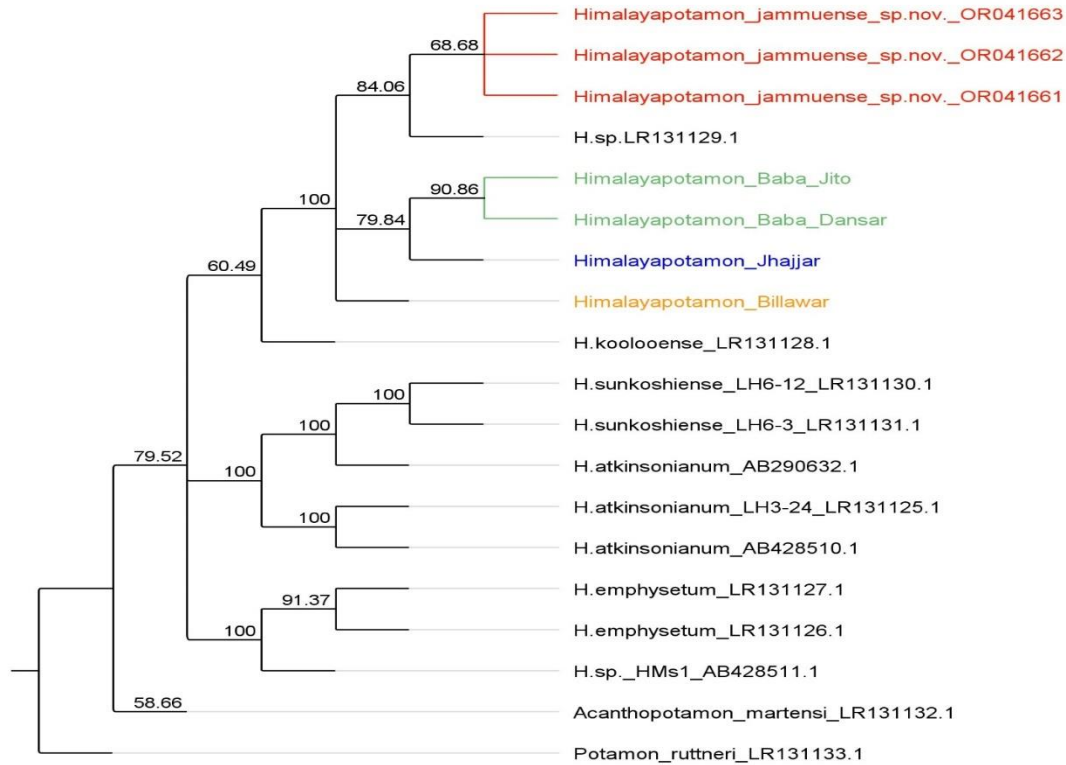


Fig. 1. BI tree of new species of freshwater crabs from Jammu region (All the species in color are first time submissions)

A summary sheet of not more than two pages under the following heads (Title, Introduction, Rationale, Objectives, Methodology, Results)

Title: Human pulmonary paragonimiasis in crab-eating communities and smear-negative suspected TB cases from some states of India (Union Territory Jammu and Kashmir)

Introduction

Pulmonary paragonimiasis is often misdiagnosed with smear-negative pulmonary tuberculosis due to overlapping clinical manifestations and radiological pictures. Studies have revealed that pulmonary paragonimiasis is one of the important public health concerns in the northeastern (NE) region of India. However, in other states of India, the public health importance of paragonimiasis is not known. Anecdotal studies have shown the presence of lung flukes in felines, canines and other animals from different states of India. However, there is no comprehensive study available on human paragonimiasis in other states of India from where lung flukes have occasionally been reported in animals. Although a few human case

reports have been published, no epidemiological or molecular evidence is available about the species of lung flukes involved. Besides, information on crab species acting as intermediate hosts is also lacking. So, it is important to survey freshwater crab-eating communities where the probability of finding foci of pulmonary paragonimiasis is high.

Rationale

Human paragonimiasis may be widespread in fresh-water crab-eating communities in India and different lung fluke species complexes may be present in different states of India. Fresh-water crabs species acting as intermediate hosts of lung flukes in India need to be identified.

Objectives

1. To detect human pulmonary paragonimiasis in fresh-water crab-eating communities of India and smear-negative pulmonary TB cases.
2. To examine local freshwater crabs for metacercarial infection due to *Paragonimus* sp.
3. To confirm lung fluke species using molecular techniques and laboratory animal experimentation.

Methodology

Study states included in the present study stations of J&K, Himachal Pradesh and Uttarakhand. The study components are listed below:

- **Active surveys:** Cross-sectional door-to-door surveys are being carried out in the selected villages for the detection of pulmonary paragonimiasis in selected fresh-water crab-eating communities (exploratory survey)
- **Passive surveys:** Suspected TB cases attending PHC/Microscopy centers in the selected villages are being screened for pulmonary paragonimiasis
- **Faunal surveys:** A faunal survey of freshwater crabs in selected study villages was performed.
- **Gene Sequencing:** Molecular confirmation of freshwater crab species was based on sequencing of two mitochondrial genes (16S rRNA gene and cox1 gene) and two nuclear genes (28S rRNA and Histone3).

Results

Summary of work done during 5 years:

- In summary, from Oct 2018 to Oct 2023, a total 7,515 number of individuals from three study states (J&K, Himachal Pradesh and Uttarakhand) were enrolled in active surveys out

of which blood samples were collected from 498 individuals. A total of 498 serum samples were tested for IgG antibodies against adult lung flukes and 0 serum samples were found to be immuno-reactive for paragonimiasis by ELISA. Similarly, in passive survey a total number of 569 blood samples were collected from all the sites put together. Of these, 0 passive samples were found to be immuno-reactive for paragonimiasis by ELISA.

- A total of 5,649 crabs have been collected from the period of Oct 2018 to Oct 2023. Molecular characterization of species new to science was done and the phylogenetic analysis showing the justification of the new species has been attached (Fig.1).
- Analysis of the crab specimens from various regions of the country has shown that there are many species of crabs that remain still unknown to science and need more taxonomic characterization efforts both on morphological and molecular levels to know the exact identify of the same.

Contributions made towards increasing the state of knowledge in the subject.

- Four new freshwater crab species have been identified both on the basis of morphological and molecular characters from J&K.
- Two new records of freshwater crabs have been found from J&K.
- For the first time four genes (two mitochondrial and two nuclear genes) of freshwater crabs have been studied from J&K.

Conclusions summarizing the achievements and indication of scope for future work.

From the survey various freshwater crabs species new to science have come to light. Though three states have been screened for the presence of parasite, various other metacercarial infections were found but no success was achieved to recover the parasite *Paragonimus* sp. either from crab species or any serum positive samples were recovered.

Science and Technology benefits accrued:

List of research publications with complete details:

- Kour, H., Langer, S., & Mitra, S. (2019). Survey status and morphometric characterization of two species of freshwater crabs from Jammu division (J&K State). *International Journal of Research and Analytical Reviews*, 6(2), 278-285.
- Dhar, M., Langer, S., & Jasrotia, R. (2021). Karyotypic analysis of freshwater crab,

Maydelliathelphusa masoniana (Henderson, 1893) from Jammu region of J&K. *Indian Journal of Ecology*, 48(3), 765-770.

- Kour, H., Langer, S., & Sharma, N. (2022). Population dynamics of *Maydelliathelphusa masoniana* Henderson 1893 from freshwater bodies of Jammu region (Jammu and Kashmir, India). *Eco. Env. & Cons.* 28 (February Suppl. Issue): pp. (S425-S435).
- Dhar, M., Langer, S., & Rajput, S. (2022). Studies on freshwater crab population as influenced by physico-chemical attributes in Gho-Manhasan stream, a tributary of river Chenab. *Eco. Env. & Cons.*, 28(1), 140-146.
- Dhar, M., Langer, S., & Gupta, C. (2022). Insights into the Habitat Choice for the Culture of *Himalayapotamon emphysetum* under Laboratory Conditions. *Biosciences Biotechnology Research Asia*, 19(4), 1019-1024.
- Dhar, M. & Langer, S. (2021). Optimization of cytogenetic protocol for chromosome preparation in freshwater crabs. *Biosc. Biotech. Res. Comm.* 14(3), 1256-1259.
- Manpower trained in the project:
 - Research Fellows (FI & PTO) = 4
 - No. of PhDs produced = 2
 - Other Technical Personnel trained = 5

Abstract: Four new freshwater crab species have been identified both on the basis of morphological and molecular characters from J&K. Two new records of freshwater crabs have been found from J&K. For the first time four genes (two mitochondrial and two nuclear genes) of freshwater crabs have been studied from J&K. From the survey various freshwater crabs species new to science have come to light. Though three states have been screened for the presence of parasite, various other metacercarial infections were found but no success was achieved to recover the parasite *Paragonimus* sp. either from crab species or any serum positive samples were recovered.

Procurement/usage of Equipment

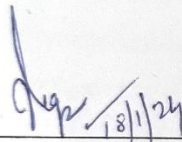
S. No	Name of Equipment	Make/Model	Cost FE/	Date of Installation	Utilization rate%	Remarks regarding maintenance/breakdown

1.	-20°C Ultra Freezer	PLF 276	4,24,000	23-05-2019	90	Has been working properly from the date of installation till now
2.	Binocular stereomicroscope with integrated camera	SZ61Tr + Magcam HD lite	3,89,000	23-05-2019	99	Has been working properly from the date of installation till now
3.	Microplate ELIZA Reader	MULTI SKAN FC	3,95,000	05-04-2019	99	Has been working properly from the date of installation till now
4.	4°C Freezer	R700-GAEV-TSC	2,98,000	27-05-2019	90	Has been working properly from the date of installation till now
5.	Compound Research Microscope	MLxi Plus + Magcam DC-5	2,76,490	28-05-2019	99	Has been working properly from the date of installation till now
6.	Micropipette	Single channel	2,49,570	15-10-2019	100	Has been working properly from the date of installation till now
7.	Computer	400G4A IO 178700 WIN 10PDV D/RW/2 .38	1,65,000	07-10-2019	100	Has been working properly from the date of installation till now
8.	Laptop	250G7 is 8 th /8GB/ ITB/15/	46,440	07-10-2019	100	Has been working properly from the date of installation till now

		W105L L6YN32 PA				
9.	Printer	128 fn Laserjet Printer HP	59,640	15-10-2019	100	Has been working properly from the date of installation till now
10.	UPS	UPS 1.0 KVA Microte k TGE 1000+	9,702	15-10-2019	100	Has been working properly from the date of installation till now

Name and signature with date

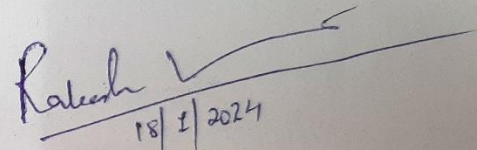
1.


18/1/24

(Principal Investigator)

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In Crab Eating Communities (ICHR)
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2.


18/1/2024

(Co-Investigator)