Template/Pro forma for Submission

NMHS-Himalayan Institutional Fellowship Grant

FINAL TECHNICAL REPORT (FTR)

NMHS Reference No.:	**GBPNI/NMHS-2018-19/HSF25-03**

Date of Submission:	2	4	1	2	2	0	2	2
	d	**d**	**m**	**m**	**y**	**y**	**y**	**y**

FELLOWSHIP TITLE

HIMALAYAN RESEARCH FELLOWSHIP 2018-19

Sanctioned Fellowship Duration: *from* (**17.12.2018**) *to* (**16.12.2021**).

***Submitted to*:**

Er. Kireet Kumar
Scientist 'G' and Nodal Officer, NMHS-PMU
National Mission on Himalayan Studies, GBP NIHE HQs
Ministry of Environment, Forest & Climate Change (MoEF&CC), New Delhi
E-mail: nmhspmu2016@gmail.com; kireet@gbpihed.nic.in; kodali.rk@gov.in

***Submitted by*:**
[*Professor Lalramliana*]
[*Pachhunga University College, Aizawl, 796001, Mizoram*]
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GENERAL INSTRUCTIONS:

1. The Final Technical Report (FTR) has to be commenced from the date of start of the Institutional Fellowship (as per the Sanction Order issued at the start of the Fellowship) till its completion. Each detail has to comply with the NMHS Sanction Order.
2. The FTR should be neatly typed (in Arial with font size 11 with 1.5 spacing between the lines) with all details as per the enclosed format for direct reproduction by photo-offset process. Colored Photographs (4-5 good action photographs), tables and graphs should be accommodated within the report or should be annexed with captions. Sketches and diagrammatic illustrations may also be given giving step-by-step details about the methodology followed in technology development/modulation, transfer and training. Any correction or rewriting should be avoided. Please give information under each head in serial order.
3. Training/ Capacity Building Manuals (with detailed contents of training programme, technical details and techniques involved) or any such display material related to fellowship activities along with slides, charts, photographs should be sent at the NMHS-PMU, GBP NIHE HQs, Kosi-Katarmal, Almora 263643, Uttarakhand. In all Knowledge Products, the Grant/ Fund support of the NMHS should be duly acknowledged.
4. The FTR Format is in sync with many other essential requirements and norms desired by the Govt. of India time-to-time, so each section of the NMHS-FTR needs to duly filled by the Fellowship Coordinator/ PI and verified by the Head of the Implementing Institution/ University.
5. Five (5) bound hard copies of the NMHS-Institutional Fellowship Final Technical Report (FTR) and a soft copy should be submitted to the **Nodal Officer, NMHS-PMU, GBP NIHE HQs, Kosi-Katarmal, Almora, Uttarakhand** *via* e-mail nmhspmu2016@gmail.com.

   The FTR is to be submitted into following two parts:

   Part A – Cumulative Fellowship Summary Report

   Part B – Comprehensive Report

   Following Financial and other necessary documents/certificates need to be submitted duly signed and verified along with Final Technical Report (FTR):

Annexure I	Consolidated and Audited Utilization Certificate (UC) & Statement Expenditure (SE), including interest earned for the last Fiscal year including th duly filled GFR-19A (with year-wise break-up)
Annexure II	Consolidated Interest Earned Certificate
Annexure III	Consolidated Manpower Certificate and Direct Benefit Transfer (DBT) Detai showing the education background, i.e. NET/GATE etc. qualified or not, Date joining and leaving, Salary paid per month and per annum (with break up a per the Sanction Order and year-wise).
Annexure IV	Details and Declaration of Refund of Any Unspent Balance as Real-Time Gros System (RTGS) in favor of NMHS GIA General
Annexure V	Details of Technology Transfer and Intellectual Property Rights developed.

NMHS-Final Technical Report (FTR) *template*

NMHS- Institutional Himalayan Fellowship Grant

DSL: *Date of Sanction Letter*

1	7	1	2	**2**	**0**	**1**	**8**
d	d	m	m	y	y	y	y

DFC: *Date of Fellowship Completion*

1	6	1	2	**2**	**0**	**2**	**1**
d	d	m	m	y	y	y	y

Part A: CUMULATIVE SUMMARY REPORT

(to be submitted by the Coordinating Institute/Coordinator)

1. Details Associateship/Fellowships

1.1. Contact Details of Institution/University

NMHS Fellowship Grant ID/ Ref. No.:	U/I ID: HSF2018-19/I-25/03
Name of the Institution/ University:	Pachhunga University College
Name of the Coordinating PI:	Prof. Lalramliana
Point of Contacts (Contact Details, Ph. No., E-mail):	lrl_zoo@yahoo.co.in

1.2 Research Title and Area Details

i.	Institutional Fellowship Title:	**HRA:** Diversity and characterization of helminth parasites of freshwater fishes and evaluation of their zoonotic potential in Mizoram, northeast India **HJRF:** Diversity and sustainable utilization of the entomopathogenic bacteria (*Xenorhabdus* and *Photorhabdus*) from Mizoram, northeast India					
ii.	IHR State(s) in which Fellowship was implemented:	**Mizoram**					
iv.	Scale of Fellowship Operation	Local:	✓	Regional:		Pan-Himalayan:	
iii.	Study Sites covered (*site/location maps to be attached*)	Attached					
v.	Total Budget Outlay (Crore):	INR **36,41,616**					

1.3 Details Himalayan Research /Project Associates/Fellows inducted

Type of Fellowship	Nos.	Work Duration	
		From	To
Research Associates	**1**	**17.12.2018**	**16.12. 2021**
Jr. Research Fellows	**1**	**17.12.2018**	**16.12. 2021**

2. Research Outcomes

2.1. Abstract (not more than 1000 words) (it should include background of the study, aim, objectives, methodology, approach, results, conclusion and recommendations based on the institutional fellowship proposal sanctioned under the NMHS).

HRA

Background: Helminth parasite infections are amongst the most common parasitic infection of humans worldwide. A large number of marine and freshwater fishes can serve as a source of medically important parasitic zoonoses. In the state of Mizoram Northeast India, a biodiversity hotspot, fishes constitute an important part of the diet and through recent survey prevalence of fish borne helminth infections have been reported especially from rural areas but there is no published record of them to date.

Objectives/ Aim: In context of Mizoram there are very few reports available on the parasitic helminth fauna of fishes. The aim of this study is to collect the available fishes from different riverine system of Mizoram and to recover and documenting potential zoonotic parasites.

Methodology: In these studies fishes were collected from the different riverine systems of Mizoram covering different districts. Fishes were then examined and the parasites recovered and processed following standard procedure of mounting and fixation and identified. Our morphological findings are further supported by DNA data using ITS2 and COI rDNA and ultrastructural Data.

Approach: The research will be based on field survey and laboratory analysis. Emphasis will be given to those fish species, which are commonly used in local traditional cuisine in selected pockets of Mizoram. The species will be identified through morphological and molecular analysis (using ITS and COI gene region). The work will ascertain the occurrence and distribution of various zoonotic helminth parasites and the prevalence of relatively common and rare groups in the fish host. The work will emphasize on the neglected study in the region which are plausible factor of human and animal diseases. The work on completion will resolve, whether or not, the fish production systems are at risk of presenting fish zoonotic parasites, and that control approaches will benefit from understanding these risk factors

Results: Twenty different species of fishes were examined for recovery of the parasite, the recovered helminth parasites include nematode, cestodes, trematodes and an acanthocephala, in which trematode was found to be the most prevalent and abundant group whereas acanthocephala was the least encountered. Among the fishes *Mastacembelus armatus* belonging to the family Mastacembelidae is the only fish which harbour three different groups of the helminth parasites

HJRF

Background: *Photorhabdus* and *Xenorhabdus* are symbiotic bacteria of entomopathogenic nematodes of the genus *Heterorhabditis* and *Steinernema* respectively. The EPNs have a cosmopolitan distribution (except Antarctica) and are utilized effectively for the biological control of insect pests with a high success rate. A total of 19 species of the genus *Photorhabdus* and 26 species of the genus *Xenorhabdus* associated with the nematodes have been identified so far When the mutualistic association infects the insect larvae, the symbiotic bacteria are known to produce broad-spectrum compounds which are lethal to the infected larvae including activity against bacteria, fungi, nematode, protozoa, and cancer cells. Meanwhile, several compounds produced by these bacterial symbionts are known to protect the insect cadaver microenvironment due to their antimicrobial, nematicidal, and insecticidal activity.

Objectives/ Aim: The aim of this study is to characterized the bacterial symbionts from Mizoram, North-east India and evaluation of their insecticidal, antifungal and antibacterial activity.

Methodology: The bacterial isolates were characterized using16S rRNA, recA and gyrB gene region. The ethyl acetate extract of bacterial isolates was tested against pathogenic bacterial strains, viz. *Escherichia coli* (ATCC 10536), *Klebsiella pneumoniae* (ATCC 10031), *Pseudomonas aeruginosa* (ATCC 10145), and *Bacillus subtilis* (ATCC 11774) using disk diffusion method. Screening of antifungal activity was conducted by placing 4 mm of fungal mycelium on petri-plate consisting of spread bacterial suspensions adjusted to 3 × 10. Insecticidal activity was conducted against *G. melonella* larvae.

Approach: The study will focus on the sustainable utilization of biodiversity by means of biological control of insect pests. The targeted species will be the entomopathogenic bacteria of the genera X*enorhabdus* and *Photorhabdus* associated with the entomopathogenic nematodes. Since the study area is a part of the world biodiversity hotspot region, the organisms distributed in the region are distinct. It is hypothesized that the bacterial species from this region are also distinct, probably high virulent novel species or strains. The pure culture of the isolated bacteria will be phenotypically and biochemically characterized and their genetic diversity will be studied base on 16S rRNA, rec A and gyr B gene. The insecticidal and antimicrobial properties will be evaluated and the promising species or strains will be documented for future use. In short, the proposed study aims to survey and identify EPB, know their potential as biocontrol agent, their insecticidal and antimicrobial properties, understand the genetic biodiversity, genetic distance among species and in between species, and generation of valid database for reducing the use of chemical pesticides.

Results: The morphological and molecular characterization shows that the isolated bacteria were *Photorhabdus hindustanensis*, *Photorhabdus namnaonensis*, *Xenorhabdus vietnamensis* and *Xenorhabdus stockiae*. This is the first report of *Photorhabdus namnaonensis* from India. Larval mortality rate was observed during 24 to 96 hours of exposure. At a concentration of 10^8 CFU/mL, 100% mortality rate was observed after 96 hours of treatment in all the isolates. The ethyl acetate extract of the isolated bacteria inhibited the growth of all the tested pathogenic bacterial strain. The inhibition of fungal growth by the isolated bacterial suspension was observed within 192 hr post inoculation.

***Conclusion*:** This study focuses on the existence and identification of symbiotic bacteria from Mizoram, an Indo-Burma biodiversity hotspot region, and details on their activity against insect pest, different pathogenic bacteria and fungi. Since, these metabolites could be a potent antibiotic, further research is required to better understand the genetic information, chemical composition, and the method of action against other microorganisms.

Recommendations: Looking at the potential inhibitory property of the bacterial isolates against pathogenic microorganisms along with their insecticidal property, we recommend further studies regarding microbial products and formulations for effective treatment of insect pests and pathogenic microorganisms.

2.2. Objective-wise Major Achievements

S. No.	Cumulative Objectives	Major achievements (in bullets points)
1	Explore diversity and identify the helminth parasites of common freshwater fishes from various localities in Mizoram	• 254 fishes belonging to three different species of fishes *Mastacembelus armatus, Xenentodon cancila* and *Glyptothorax maceriatus* which were commonly consumed by the locals were collected from different river sites.
2	Morphological and molecular characterization (using ITS and COI region) of the parasites species	• The recovered helminth parasites include nematode, cestodes, trematodes and an acanthocephala, in which trematode was found to be the most prevalent and abundant group whereas acanthocephala was the least encountered. • The pseudophyllidean cestodes recovered belongs to the genus *Senga* sp. • An Acanthocephalan species belonging to the genus *Neoechinorhynchus* sp. • The Trematodes collected were identified as Digenean belonging to the genus *Prosorhynchoides aspinosus* n.sp., *Plagioporus* sp. and *Phyllodistomum* sp. *Bucephalus* and *Podocotyloides* sp. And were recovered from *Glyptothorax maceriatus*, *M. Armatus* and *Xenentodon cancila* and *Pseudoluguvia spicula.* • The nematodes recovered belong to the genus *Spinitectus* sp., *Paracamallanus* sp., and *Capillaria* sp.

3	Assess the role of fishes in disseminating plausible helminthes in human and other animals.	• There is lot of variation in the prevalence of parasite among fishes collected from different river sites, but it might be interesting to note that the river Tuirial which flows near the human settlement, and also near the city dumping ground, tends to harbour more diverse group of parasites. With mostly all the three group of parasites present in a single host. • Trematodes were found to be the most common helminth parasites infecting the four different fish host followed by nematodes which infect three different hosts whereas cestodes and *Acanthocephala* infected two different hosts.
4	To isolate, identify and study the biochemical activity of entomopathogenic bacteria associated with *Steinernema* spp. and *Heterorhabditis* spp. of Mizoram, NE India.	• Bacterial symbionts from their symbiotic nematodes were isolated from different sites within the proposed study area *ie*. Mizoram. • Morphological and biochemical characterization were analysed.
5	To study the genetic diversity and molecular phylogeny of isolated bacteria using 16SrRNA, rec A and gyr B gene region.	• 16S rRNA, recA and gyrB genes of the isolates were amplified and the products were sequenced and analysed. • The developed sequences were submitted to NCBI Gen Bank
6	To evaluate the insecticidal and antimicrobial activity of the isolated bacteria	• Insecticidal activity against *G. melonella* was evaluated. • Antibacterial activity against several pathogenic bacteria was evaluated. • Antifungal activity against *F. oxysporum, F. solani* and *C. albicans* was evaluated.

2.3. Outputs in terms of Quantifiable Deliverables*

S. No.	Quantifiable Deliverables*	Monitoring Indicators*	Quantified Output/ Outcome achieved	Deviations made, if any, and Reason thereof:
1	The clear picture of fish-borne helminth zoonotic parasites occured in riverine and cultivated fishes in Mizoram, posing potential risk to human and other animals health will be documented.	• Fishes were collected from the different riverine systems of Mizoram covering different districts. • Twenty different species of fishes were examined for recovery of the parasite.	• One zoonotic organism was identified. • The recovered helminth parasites include nematode, cestodes, trematodes and an acanthocephala, in which trematode was found to be the most prevalent and abundant group whereas acanthocephala was the least encountered. • Besides fishes, helminth parasites such as metacercaria of some digenea and Aspidogastrea were recovered from Crab and snails from the river of Mizoram, which are yet to be identified.	
2	Up-to-date database will be generated on various fish parasite species from Mizoram; and the genetic variability studies can help in evaluating conservation status of related fish species in Mizoram.	Data were recorded on the prevalence and intensity of helminth infection in piscine hosts and the data were analyzed following Bush *et al*., (1997).	• There is lot of variation in the prevalence of parasite among fishes collected from different river sites, but it might be interesting to note that the river Tuirial which flows near the human settlement, and also near the city dumping ground, tends to harbour more diverse group of	

			parasites. With mostly all the three group of parasites present in a single host.	
3	Resolving, whether or not, the fish production systems are at risk of presenting fish zoonotic parasites, and that control approaches will benefit from understanding these risk factors	• The piscine hosts collected were examined for helminth infections. • Their external body surface and internal organs were examined for the presence of parasites. • The helminth parasites recovered were counted, stretched in warm water, flattened and processed further following standard procedures of fixation, preservation and staining.	• The helminth communities of these freshwater fishes in Mizoram are species-poor, and that, considerable proportion of fish from the region is uninfected or lightly infected.	
4	Promoting green technology by introducing EPB as a component of Integrated Pest management programme in Mizoram and selection of high virulence, persistence, reproducing capacity etc. species/strains for a good biological control agent.		• The bacterial isolates were highly virulent to the selected pest. • This experimental results highlight the potential activity of the isolates to a certain degree and its delivery methods in the field conditions needs to be investigated either through formulations or the secondary products of the isolates alone.	
5	Exact identification of the species will provide documentation of endemic species and	Morphological molecular analysis was conducted for identification of fish parasites and	• Four different species of symbiotic bacteria were isolated. • Variations of activities including degree of	

	the novel species/strains of the region rendering effective use of the bacteria and its host as biological control programme and conservation programmes.	symbiotic bacteria	inhibition were observed among the isolates.	
6	Availability of reference DNA barcodes along with well identified vouchers specimen will be an asset for future biodiversity research, conservation, ecology, pest management and genetic enhancement programmes	16S rRNA, gyrB and recA gene regions were used for identification of the isolated symbiotic bacteria	• Molecular characterization using the selected primer sequences revealed that *P. namnaonensis* was first reported in India. All the developed sequences were submitted to NCBI GenBank for documentation and future references.	

(*) As stated in the Sanction Letter issued by the NMHS-PMU.

2.4. Strategic Steps with respect to Outcomes (in bullets)

S. No.	Particulars	Number/ Brief Details	Remarks/ Enclosures
1.	New Methodology developed:	Nil	
2.	New Models/ Process/ Strategy developed:	Nil	
3.	New Species identified:	1	Accepted
4.	New Database established:	5	New database submitted to NCBI GenBank
5.	New Patent, if any:	Nil	
	I. Filed (Indian/ International)		
	II. Granted (Indian/ International)		
	III. Technology Transfer (if any)		
6.	Others, if any:	Nil	

3. Technological Intervention

S. No.	Type of Intervention	Brief Narration on the interventions	Unit Details (No. of villagers benefited / Area Developed)
1.	Development and deployment of indigenous technology	Nil	
2.	Diffusion of High-end Technology in the region	Nil	
3.	Induction of New Technology in the region	Nil	
4.	Publication of Technological / Process Manuals	Nil	
	Others (if any)	Nil	

4. New Data Generated over the Baseline Data

S. No.	New Data Details	Existing Baseline	Additionality and Utilisation of New data (*attach supplementary documents*)
1.	9 Species of fish parasites were reported	No previous record within the study area. All sequences were submitted to NCBI GenBank	**This finding will help in better identification and will provide valid documentation for further studies.**
2.	New species was reported	Accepted and published	
3.	12 species of bacteria were reported. Among the reported species, one is the first report from India	**No previous record within the study area**. All sequences were submitted to NCBI GenBank	

5. Linkages with Regional & National Priorities (SDGs, INDC, etc.)/ Collaborations

S. No.	Linkages /collaborations	Details	No. of Publications/ Events Held	Beneficiaries
1.	Sustainable Development Goals (SDGs)	Nil		
2.	Climate Change/INDC targets	Nil		
3.	International Commitments	Nil		
4.	National Policies	Nil		
5.	Other collaborations	Nil		

6. Financial Summary (Cumulative)*

Consolidated and Audited
Utilization Certificate (UC) and Statement of Expenditure (SE)

For the Period: 17/12/2018 to 16/12/2021

1.	Title of the fellowship/Scheme:	**NMHS Research Fellowship**
2.	Name of the Principal Investigator & Organization:	**Prof. Lalramliana**
3.	NMHS-PMU, G.B. Pant National Institute of Himalayan Environment, Kosi-Katarmal, Almora, Uttarakhand Letter No. and Sanction Date of the Fellowship:	GBPNI/NMHS-2018-19/HSF25-03 Dt. 17. 12. 2018
4.	Amount received from NMHS-PMU, G.B. Pant National Institute of Himalayan Environment, Kosi-Katarmal, Almora, Uttarakhand during the fellowship period (Please give number and dates of Sanction Letter showing the amount paid):	**35,12,518**
5.	Total amount that was available for expenditure (including commitments) incurred during the fellowship period:	**35,12,518**
6.	Actual expenditure (excluding commitments) incurred during the fellowship period:	**34,28,049**
7.	Unspent Balance amount refunded, if any (Please give details of Cheque no. etc.):	
8.	Balance amount available at the end of the fellowships:	**99,718**
9.	Balance Amount:	**99,718**
10.	Accrued bank Interest:	**15,249**

Certified that the expenditure of **Rs. 34,28,049 (Thirty-four lakhs twenty-eight thousand and forty-nine only)** mentioned against Sr. No. 6 was actually incurred on the fellowship/scheme for the purpose it was sanctioned.

(Signature of Principal Investigator)
Professor
Department of Zoology
Pachhunga University College
Mizoram University

(Signature of Registrar/Finance Officer)
Section Officer (Fin)
Pachhunga University College
Aizawl : Mizoram

(Signature of Head of the Institution)
Principal
Pachhunga University College
Aizawl : Mizoram

FOR AKAS & ASSOCIATES LLP
Chartered Accountants
Partner
AJAY JOSHI
M. No. 98017
21/12/22

PACHHUNGA UNIVERSITY COLLEGE ★ AIZAWL ★

Unique Document Identification Number (UDIN) for this document is 22098017BFWDOD3892

Sanction No. and Date: **GBPNI/NMHS-2018-19/HSF25-03/154/488** **Date: 17.12.2018**

1. Total outlay of the Fellowship: **Rs. 36,41,616 (Thirty-Six Lakh Forty-One Thousand Six Hundred Sixteen)**

2. Date of Start of the Fellowship : **17/12/2018**

3. Duration : **03 yrs**

4. Date of Completion : **16/12/2021**

a) Amount received during the fellowship period : **35,12,518**

b) Total amount available for Expenditure : **35,12,518**

S. No.	Budget head	Amount received	Expenditure	Amount Balance/ excess expenditure
1	Salaries	23,22,756	22,23,720	99,036
2.	Permanent Equipment purchased	Nil	Nil	Nil
3	Contingency (including domestic travel)	8,70,000	8,69,318	682
4.	Expendables/Consumables	Nil	Nil	Nil
5	Institutional charges	3,19,762	3,19,762	0
6	Accrued bank Interest	15,249	**15,249***	0
7	**Total**	**35,27,767**	**34,28,049**	**99,718**

Certified that the expenditure of **Rs. 34,28,049 (Thirty-four lakhs twenty-eight thousand and forty-nine only)** mentioned against Sr. No.12 was actually incurred on the fellowship/ scheme for the purpose it was sanctioned.

* Interest **Rs. 15,249** deposited back to the Govt.

(Signature of Principal Investigator)

Professor
Department of Zoology
Pachhunga University College
Mizoram University

(Signature of Registrar/Finance Officer)

Section Officer (Fin)
Pachhunga University College
Aizawl : Mizoram

(Signature of Head of the Institution)

Principal
Pachhunga University College
Aizawl : Mizoram

FOR AKAS & ASSOCIATES LLP
Chartered Accountants
FRN: 132876N
Partner
AJAY JOSHI
M. No. 98017
21/12/22

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Unique Document Identification Number (UDIN) for this document is 22098017BFWDOD3892

S. No.	Parameters	Total (Numeric)	Attachments* with remarks
1.	IHR State(s) Covered:	**1**	Mizoram
2.	Fellowship Site/ LTEM Plots developed:		
3.	New Methods/ Model Developed:	**Nil**	
4.	New Database generated:	**18**	Submitted to NCBI GenBank
5.	Types of Database generated:	**Sequences**	Details were given in Comprehensive Report
6.	No. of Species Collected:		
7.	New Species identified:	**1**	*Prosorhynchoides aspinosus* (Accepted and Published)
8.	Scientific Manpower Developed (PhDs awarded/ JRFs/ SRFs/ RAs):	**2**	1 RA and 1 SRF
9.	No. of SC Himalayan Researchers benefited:	**Nil**	
10.	No. of ST Himalayan Researchers benefited:	**2**	HJRF is registered for Ph. D programme under Mizoram University
11.	No. of Women Himalayan Researchers empowered:	**2**	
12.	No. of Knowledge Products developed:		
13.	No. of Workshops participated:	**4**	
14.	No. of Trainings participated:	**2**	
15.	Technical/ Training Manuals prepared:	**1**	1 week Training on Microbial handling and DNA extraction for B. Sc and M.Sc students at Pachhunga University College
	Others (if any):		

***** Please attach the soft copies of supporting documents word files and data files in excel.

8. Knowledge Products and Publications*

S. No.	Publication/ Knowledge Products	*Number*		*Total Impact Factor*	*Remarks/* Enclosures**
		National	International		
1.	Journal Research Articles/ Special Issue (Peer-reviewed/ Google Scholar)	3	1	0.719+2.106	Enclosed
2.	Book Chapter(s)/ Books:	Nil			
3	Technical Reports/ Popular Articles	Nil			

S. No.	Publication/ Knowledge Products	*Number*		*Total Impact Factor*	*Remarks/* Enclosures**
		National	International		
.					
4.	Training Manual (Skill Development/ Capacity Building)	Nil			
5.	Papers presented in Conferences/ Seminars	1	3		Enclosed
6.	Policy Drafts (if any)	Nil			
7.	Others (specify)	Nil			

List of publications:

1. Chenkual Malsawmtluangi, Lalramliana, A new species of *Prosorhynchoides* Dollfus, 1929 (Digenea: Bucephalidae) from *Xenentodon cancila* Hamilton, 1822 in Mizoram, Northeast India, Parasitology International, Volume 92, 2023, 102690, ISSN 1383-5769, https://doi.org/10.1016/j.parint.2022.102690.
2. Malsawmtluangi, C. and Lalramliana: Studies on the distribution and diversity of helminth infection in *Xenentodon cancila* (Hamilton, 1822) in Mizoram, Northeast India. J. Environ. Biol., 41, 832-839 (2020). http://doi.org/10.22438/jeb/4(SI)/MS_1901
3. Lalramchuani, M., H.C. Lalramnghaki, R. Vanlalsangi, E. Lalhmingliani, Vanramliana and Lalramliana: Characterization and screening of antifungal activity of bacteria associated with entomopathogenic nematodes from Mizoram, North-Eastern India. J. Environ. Biol., 41, 942-950 (2020). http://doi.org/10.22438/jeb/4(SI)/MS_1913
4. Lalramchuani, M., Lalramnghaki, H. C., & Lalhmingliani, E: Molecular Characterization and Antibacterial Activities of *Photorhabdus* and *Xenorhabdus* from Mizoram, North-East India. J Pure Appl Microbiol., *17*, 1560 - 1577 (2023) https://doi.org/10.22207/JPAM.17.3.18

List of conferences and seminars:

1. **Poster Presented** in 'International Conference on recent advances in animal sciences'. Organised by Dept of Zoology, Pachhunga University College, from 6-8 November, 2019.
   (Received best poster Award: On the topic **'Studies on the distribution and diversity of helminth infection in *Xenentodon cancila* from different rivers of Mizoram, Northeast India'.)**

2. Oral presentation at 'International Conference on Recent Advances in Animal Sciences'-2019 Organised by Dept of Zoology, Pachhunga University College, from 6-8 November, 2019. on the topic- **'Evaluation of the larvicidal activity of two locally isolated Entomopathogenic Bacteria (*Xenorhabdus* and *Photorhabdus*) against *Galleria mellonella* L'.**

3. Poster Presentation at 'TROPACON N.E 2022 organised by Indian Academy of Tropical Parasitology, NE Chapter and Zoram Medical College, from 9-10 November, 2022 on the topic '**Antibacterial activity of *Photorhabdus* and *Xenorhabdus* isolated from Mizoram, North-East India'.**

4. Poster presentation at 'International Conference on Biodiversity and Conservation (ICBC) organized by Dept of Zoology and Fishery Science, St. Anthonys College, Shillong, from 14-15 December 2022 on the topic '**Antagonistic Potential of *Photorhabdus* and *Xenorhabdus* against *F. solani* from Mizoram, North- East India'**

9. Recommendation on Utility of Research Findings, Replicability and Exit Strategy

9.1 Utility of the Fellowship Findings

S. No.	*Research Questions Addressed*	*Succinct Answers (within 150–200 words)*
1.	What are the different species of helminths that infects fishes of Mizoram?	The recovered helminth parasites include nematode, cestodes, trematodes and an acanthocephala, in which trematode was found to be the most prevalent and abundant group whereas acanthocephala was the least encountered. There is lot of variation in the prevalence of parasite among fishes collected from different river sites, but it might be interesting to note that the river Tuirial which flows near the human settlement, and also near the city dumping ground, tends to harbour more diverse group of parasites. With mostly all the three group of parasites present in a single host. 9 different helminth parasites were reported in which only *Capillaria* sp. was found to be Zoonotic. A new species of Digenea *Prosorhynchoides aspinosus* n.sp was reported .
2.	What are the helminth parasites of fishes which are zoonotic to human and other animals?	*Capillaria* sp. a nematode belonging to the genus recovered parasite which is zoonotic.
3.	Is there any biological control agents present in Mizoram?	So far, chemical control agents were mostly used for treatment of pests and pathogenic microbes.
4.	Can these microbes be successfully utilized for insect pest control?	The activity of these isolated organisms against insect pest provides information regarding the potential of the isolates and further formulation and detail analysis needs to be carried on to make it available for field applications.
5.	Is there any valid documentation of beneficial symbiotic microbes in Mizoram?	Since the study area is a part of the world biodiversity hotspot region, the organisms distributed in the region are distinct with different degree of activities. Therefore, it is recommended to cover more areas for documentation of these kinds of microorganisms. The information provided in this study will pave

		the way for identification and further analysis of certain beneficial microorganisms

9.2 Recommendations on Replicability and Exit Strategy:

Particulars	Recommendations
Replicability of Fellowship, if any	
Exit Strategy:	• During the survey besides the helminth parasites collected from freshwater fishes we have encountered many parasites from freshwater crab and snails such as digenea, Aspidogastrea and certain type of cercaria which further need to be thoroughly analysed and identified and larger area still need to be covered as many areas could not be covered for collection of specimens due to covid restrictions. • The present study emphasizes the occurrence and identification of symbiotic bacteria from Mizoram, North-east India, an Indo-Burma biodiversity hotspot region and provides information regarding the capability of their activity towards various pathogenic bacteria and fungi including insect pest. • However, detailed analysis and studies need to be carried on to increase the knowledge of the exact genetic information, composition of chemical compounds, and mode of action against insect pest and further formulation and detail analysis needs to be carried on to make it available for field applications

PACHHUNGA UNIVERSITY COLLEGE AIZAWL

(NMHS FELLOWSHIP COORDINATOR)
(Signed and Stamped)

(HEAD OF THE INSTITUTION)
(Signed and Stamped)
Principal
Pachhunga University College
Aizawl : Mizoram

Place: Aizawl

Date: 28/11/2022

PART B: COMPREHENSIVE REPORT (including all sanctioned positions of Researchers)

Based on the Fellowship Proposal submitted/approved at the time of sanction, the co-ordinating Principal Investigator shall submit a comprehensive report including report of all individual researchers.

The comprehensive report shall include an **Executive Summary** and it should have separate chapters on (1) **Introduction** (2) **Methodologies, Strategy and Approach** (3) **Key Findings and Results** (4) **Overall Achievements** (5) **Impacts of Fellowship in IHR** (6) **Exit Strategy and Sustainability** (7) **References/ Bibliography** and (8) **Acknowledgements** (It should have a mention of financial grant from the NMHS, MoEF&CC).

Further, description of Technical Activities, List of Trainings/ Workshops/ Seminars with details of trained resources, list of New Products developed under the fellowship, Manual of Standard Operating Procedures (SOPs) developed, Technology developed/Transferred etc should be enclosed as Appendix.

Report (hard copy) should be submitted to:

Er. Kireet Kumar
Scientist 'G' and Nodal Officer, NMHS-PMU
National Mission on Himalayan Studies (NMHS)
G.B. Pant National Institute of Himalayan Environment (GBP NIHE)
Kosi-Katarmal, Almora 263643, Uttarakhand

Report (soft copy) should be submitted at:

E-mail: nmhspmu2016@gmail.com; kireet@gbpihed.nic.in; kodali.rk@gov.in

PART B: COMPREHENSIVE REPORT

EXECUTIVE SUMMARY

The Executive Summary of the fellowship should not be more than 3–5 pages, covering all essential features in precise and concise manner as stated in Part A (Cumulative Fellowship Summary Report) and Part B (Comprehensive Report).

Fellowship Report No.: ***n of N*** *(n = Sequential number; N= Total no. of fellowships granted to the Institute/ University)*

Researchers Details

Type of Fellowship (HRA/HJRF/HJPF)	Name of Himalayan Researcher	Date of Joining	Date of Resignation**	Research Title	Name of the PI & Designation
HRA	Dr. C. Malsawmtluangi	07/12/2018		Diversity and characterization of helminth parasites of freshwater fishes and evaluation of their zoonotic potential in Mizoram, northeast India	Prof. Lalramliana
HJRF	Mary Lalramchuani	17/12/2018		Diversity and sustainable utilization of the entomopathogenic bacteria (*Xenorhabdus* and *Photorhabdus*) from Mizoram, northeast India	Prof. Lalramliana

*If the appointed researcher resigned in the mid of the fellowship duration, then also mention the name of the Himalayan researcher who carried forward the fellowship.

PART B: COMPREHENSIVE REPORT

(HRA)

Title: Diversity and characterization of helminth parasites of freshwater fishes and evaluation of their zoonotic potential in Mizoram, northeast India

1 INTRODUCTION

Fishes constitute an important part of the local delicacies and have been extensively used as a source of protein, especially for the rural poor, in Mizoram, Northeast India. However, fishes could serve as hosts to a number of helminth parasites, some of which could even turn out to be potential zoonotic parasites. Helminths cause infection and diseases both in freshwater and marine fishes, their importance being related directly to the fish that may affect the general public health (Hoffman, 1967). Certain helminth parasites are a matter of major public health concern (Yooyen *et al*., 2006). In addition, it may also cause economic loss to farmers, as parasites also compete for food with the fish host, thus, depriving fish of essential nutrients and inhibiting growth leading to morbidity and mortality (Khalil and Polling, 1997). Many of the parasites, particularly trematodes, are also of zoonotic importance.

According to Ko (1995), diseases transmitted by fish have become more widely distributed and have greater economical and medical impacts than recognized earlier. Consumption of raw or under cooked or processed fish can be the main source of infections for humans, with number of cases being reported from different geographical regions (Park *et al*., 2009). WHO (1995) has estimated that more than18 million people are currently infected with fish-borne trematodes alone and many more are at risk. More than 30,000 helminth parasites have been estimated to infect fishes, out of which many are known to cause serious menace to their hosts. Dependency of the parasite on its host and the exploitation of the fishes by the helminth parasites provide a useful research model in the field of ecology and evolutionary biology. Fishes are infected with four groups of helminths: Cestoda, Nematoda, Trematoda and an Acanthocephala. Studies on the helminth parasitic spectrum in freshwater fishes have been done in different parts of the world. In India many workers have also worked on the helminth parasites of both freshwater and marine fishes describing newer species and have made further advancement in this field using the various molecular biological tools, (Dayal, 1949; Srivastava, 1982; Dhole *et al*., 2010). Abidi (2002) has compiled a bibliography of fish pathogens of helminthic origin in India. In the context of Northeast India, works on this field have been meager, and very limited reports are available (Shomorendra *et al.*, 2005; Tandon *et al*., 2005; Binky *et al*., 2011; Koiri and Roy 2016).

In Mizoram, no work has been done to explore the diversity of helminths infecting freshwater fishes. Among many freshwater fishes, predatory fish species harbor a greater diversity and abundance of helminths, compared to herbivorous and planktivorous species (Luque and Poulin, 2004). Keeping all these different aspects in mind, the present studies was undertaken to find out the helminth parasitic spectrum of the fishes from the different rivers of Mizoram.

1.1. Background

In recent years, diseases transmitted by fish have become more widely distributed, and have greater economical and medical impacts (Ko, 1995). The World Health Organization (1995) has estimated that the number of people currently infected with fish-borne are at large and the trematodes alone exceeds 18 million. The worldwide number of people infected with small liver flukes only exceeds 45 million and many more are at risk (Keiser and Utzinger, 2009). More than

30,000 helminth parasites have been reported to infect fishes and many of them are harmful to their hosts. In Southeast Asia, there is evidence that the greatest risk factor for humans, the consumption of raw or improperly cooked or processed fish, is increasing in some regions, facilitated partly by population migrations and partly by commercial provision of these products (World Health Organization, 2004). Because of their complex life cycles, parasites are indicative of many different aspects of their host's biology, such as host diet, migration, recruitment, population distinctness, and phylogeny (Williams et al., 1992). A great diversity of parasitic organisms *viz*. protozoans, arthropods and helminthes, besides microbes, are important in fish all over the world (Hoffman, 1967). Monogenetic trematosdes, *Dactylogyrus* spp and *Gyrodactylus* spp are perhaps the most important and have been reported to cause extensive damage in carps and catfishes (Tripathi, 1957). Metacercarial infection in fishes is the main source of disease with subsequent economic loss (Paperna, 1991, 1995). Monogenoidean parasites are the most ubiquitous and abundant parasites in the aquatic environment (Ivona, 2004). The helminth parasite fauna of freshwater fishes, its frequency and distributon have been studied in many parts of the world (Choudhury and Dick, 2000; Nelson and Dick, 2002; Madhavi, 2003; Hernandez *et al.*, 2007; Popiolek and Kotusz, 2008; Alam *et al.*, 2010; Leon *et al.*, 2010; Maldonado *et al.,* 2020.). From neighbouring country such as Bangladesh Zaman *et al*. (1986), Khanum (1994), Khanum and Zaman (2000), Khanum and Farhana (2002), etc. worked on parasitic fauna of fresh water fishes. Hoque *et al*. (2006) reported two monogenean and one cestode from *Mystus aor* where they reported *Bucephalopsis karvei* (a synonym of *Prosorhynchoides karvei*) from *X. cancila*. From Pakistan Rafique *et al*., 2002 has reported on the presence of nematode *Rhabdocona magna* from *Mystus vittatus*. Abro *et al*., 2019 also reported nematode and trematode from *Wallago attu*, *Mystus cavacius*, *M. armatus* and *Cirrhina reba*.

1.2. National Status:

The states of North East India are blessed with a diversified and rich variety of fish fauna that has been largely documented (Mahapatra *et al.,* 2002). Sen (2003) updated the list of ichthyofauna of northeastern India with 165 species belonging to 85 genera under 31 families and 9 orders. Kar and Sen (2007) revealed the occurrence of bewildering diversity of fishes in Mizoram, Tripura and barak drainage. Of these, the rivers in Mizoram revealed 42 species in river Tuirial, 42 species in river Kolodyne, 31 species in river Karnafuli, 25 species in river Mat, 36 species in river Tlawng, nine species in river Tuirini, 14 species in river Serlui and 23 species in river Tuivai.

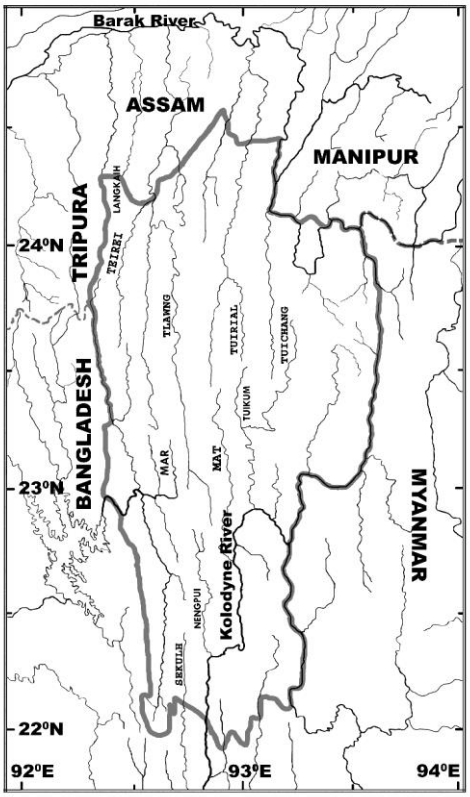


Fig 1: Map showing different collection sites

In India, many workers have reported on the pathogenicity caused to the piscine hosts by parasites and a large number of helminth parasites have been reported and described from the sub-continent, parasitizing culturable freshwater fishes (Abidi, 2002). Studies on helminths (excluding monogenoids) were started from the middle of the 19th century by some workers who came here on medical or military assignments from foreign countries (Gupta 1984). Pioneer Helminthologists have been documenting the diversity of fish parasites, most of which comprises descriptions of new taxa,

ecological aspects, the locomotion and mode of attachment, the life cycle, and pathology or, more recently, their molecular aspects from different parts of the country particularly: GD Bhalerao (1930 to 1940s); GS Thapar (1930 to 1950s); MN Datta (1930 to 1960s); BS Chauhan (1940 to 1950s); TD Soota (1950 to 1980s); PD Gupta (1960 to 1980s); CB Srivastava (1960 to 1990s); KC Pandey (1968 to present); Y Chaturvedi (1970 to 1980s); M Hafeezullah (1970 to 1990s); QH Baqri (1970 to 1990s); RK Ghosh (1980s) and MS Jayrajpuri (1980 to present). In the last three decades several workers, studied the helminth fauna of fish hosts describing many newer species or records from India and made further advancements in this field. (Mehra, 1980; Srivastava, 1982; Soota, 1983; Chakrabarti and Dutta, 2006). Soota and Ghosh (1977) conducted a sporadic faunistic survey of parasites in fishes of Meghalaya and reported the occurrence of 13 species of trematodes in them. In another study, a rich diversity of caryophyllidean cestode parasites was reported to be occurring in siluroid fishes of the region (Chakravarty and Tandon, 1988; Tandon *et al*., 2005). Recently Thapa *et al*., 2011 has reported the presence of two monogeneans from Cypriniformes, Channiformes and Siluriformes in Meghalaya. In India many workers have also worked on the helminth parasites of both freshwater and marine fishes describing newer species and have made further advancement in this field using the various molecular biological tools, (Dayal, 1949; Srivastava, 1982; Dhole *et al*., 2010). Abidi (2002) has compiled a bibliography of fish pathogens of helminthic origin in India. In the context of Northeast India, works on this field have been meager, and very limited reports are available (Shomorendra *et al.*, 2005; Tandon *et al*., 2005 Binky *et al*., 2011; Jyrwa et al., 2014; Koiri and Roy, 2016; Zimik and Roy, 2019). Kundu *et al.* (2015) reported that among the helminth parasites collected from *C. punctata* in West Bengal, an Acanthocephalan group showed minimum prevalence. Contrastingly, Ningthoukhongjam *et al*. (2015), from Manipur, reported that the Acanthocephalan infection in *C. striata* was as high as 100%. Gautam *et al*. (2018) reported that an Acanthocephalan, *Pallisentis* sp., was the most prevalent and abundant group in *Channa* sp. (46.5% in *C. punctata* and 59.11 % in *C. striata*). Mukesh and Gambhir (2016) have also reported a new species *Plagioporus* (*Caudotesti*) *minutus* in loach, *Schistura manipurensis* from Manipur.

Recently DNA techniques utilizing genetic markers in nuclear ribosomal DNA (rDNA) and mitochondrial DNA have been employed to resolve taxonomic issues related to various helminthic parasites (Blair *et al*., 1996). The second internal transcribed spacer (ITS2) of rDNA and the cytochrome c oxidase subunit I (COI) gene of mitochondrial DNA have been proven to be particularly valuable in the context (Blair *et al*., 1997). Thus, molecular techniques are increasingly being used for diagnosis, epidemiology, pathogenesis, taxonomy (Vohra, 2001). Recently, considerable progress has been achieved in studies on the phylogeny of tapeworms (Eucestoda) and new hypothesis based on morphological, life cycle, ultrastructure and molecular data have been proposed (Hoberg *et al*., 1997, 1999; Justine, 1998; Mariaux, 1998). As observed, ITS-1 and ITS-2 of rDNA provided trustworthy markers for molecular systematic along with mitochondrial DNA (Huang *et al*., 2004; Razo-Mendivil *et al*., 2010; Miura *et al*., 2005; Maurelli *et al*., 2007; Al-Kandari *et al*., 2011; Caffara *et al*., 2011; Amor *et al*., 2011; Huang *et al*., 2012; Bott *et al*., 2013; Presswell *et al*., 2014).To date, sequence data of only a few members of the orders parasitising predominantly teleost fish and reptiles, i.e. the Caryophyllidea, Pseudophyllidea and Proteocephalidea, are available.

1.3. Study Area

The state of Mizoram covers an area of 21,087 sq kms. Mizoram shares its geographical boundaries with Assam, Manipur and Tripura that extends over 123 kms, 95 kms and 66 kms respectively. It has a total of 630 miles of international border with Myanmar and Bangladesh. The terrain of Mizoram is hilly and there are a number of valleys, rivers and lakes in the state. It has the most variegated hilly terrain in the northeastern part of India. The state of Mizoram covering an area of 21,081 km^2 with maximum height of 2,743.90 approx is situated in the southern part of northeastern India, bordering Bangladesh in the south-west and Myanmar in the east having the Tropic of Cancer passing through it. Mizoram is under the influence of southwest monsoon and receives heavy rainfall during the months of May to September. Rainfall replenishes both Ground and Surface water. The surface water is found distributed in numerous streams and rivers flowing through the hilly terrain of the State which is the chief source of water for the people since underground water is not easily accessible due to hilly terrain. The climatic disturbances in the Bay of Bengal has a profound influence on the intensity of rainfall received. Heavy rains start in the month of June and continue up to August (RRC, 2019).

There are total of 15 major rivers in Mizoram, out of which Tuivawl, Tuivai, Tuirini, Tlawng, Tut and Teirei flow northward and ultimately confluence with Barak River of Mizoram valley. Mat, Tuichang, Khawchhaktuipui, Tiau and Chhimtuipui (Kolodyne) flow towards south. The remaining rivers namely Tuichawng, De and Khawthlangtuipui flow to the west. Most of the rivers in Mizoram originate in the central part of the state and flow either northerly or southerly creating deep gorges between the N-S trending hill ranges. The drainage system in Mizoram comprises of three drainages namely, Barak (Ganga Brahmaputra basin), Karnaphuli and Kolodyne basin (RRC, 2019). The rivers Tlawng (with its tributaries Teirei and Tut), Tivawl, Tuirial, Langkaih and Tuivai; drain the northern part of the region and ultimately fall into Barak River. The rivers Chhimtuipui on the east along with its tributaries Mat, Tuichang, Tiau and Tuipuidrains the southern hills; Khawthlangtuipui with its tributaries Kawrpui, Tuichawng,Phairuang, Kau and De drains the western region; Tiau and Chhimtuipui; forms boundary with Myanmar in the east and south.
All the rivers in Mizoram are monsoon fed and attain maximum volume in the monsoon and post monsoon seasons.

Lalchingpuii et al., (2011 b) studied sulphate, phosphate-P and nitrate-N contents in Tlawng river; Lalparmawii (2012) conducted analysis of water quality and biomonitoring of Tuirial river in the Vicinity 12 of the Hydel Project; Thasangzuala and Mishra (2014) Lalchhingpuii et al., (2011 a) studied the water quality of Tlawng river and reported high DO content in winter months. The total hardness and calcium hardness values of the water samples were within the WHO standards. In case of magnesium, 1.9 % of samples were higher than the permissible limit. Except for a few months, most of fluoride content was within the permissible limit. Lalparmawii and Mishra (2012) studied the water quality of Tuirial river taking into account the chemical parameters like pH, DO and BOD. The study revealed that the water was unpolluted but regular monitoring was required to assess the water quality.

This study area has been selected as the fish fauna in the state of Mizoram is diverse as three drainage system ran through it and so, far no work had been done on the spectrum of helminth parasites of fishes in Mizoram and no information is available in this regard. The collection site is depicted in the Map given below (Fig: 1).

2 METHODOLOGIES, STARTEGY AND APPROACH

2.1. Recovery of Parasites

- A survey and collection of fishes was made on monthly basis from March 2019 – till date from rivers of Mizoram covering 8 districts of Mizoram.
- The fishes collected include 20 different species of freshwater fishes
- The piscine hosts were collected with fishing net and brought to the laboratory in a container. Specimen collected were examined for helminth infections.
- Their external body surface and internal organs were examined for the presence of parasites.
- The helminth parasites and covered were counted, stretched in warm water, flattened and processed further following standard procedures of fixation, preservation and staining etc. as detailed below.

2.2. Light Microscopy:

Trematodes, Cestodes and Acanthocephala:

- Freshly recovered parasites were washed in saline solution and gently flattened between a glass slide and a cover slip and fixed overnight in 70 % ethyl alcohol.
- Whole mount preparations were made by staining in Borax carmine or Meyer's carmalum, dehydrated through ascending grades of alcohol, cleared in methyl benzoate and finally mounted in Canada balsam.

Nematodes:

- The recovered worms were stretched and fixed in warm 70 % alcohol.
- The alcohol-fixed worms were cleared in ascending grades of glycerine till pure glycerine and finally double mounted using Kaiser's glycerine jelly.
- The permanent slide was observed and studied under Motic ecoline microscope, generic identification of the parasites was done following standard reference works.
- For taxonomic identification of parasites, standard reference works of Yamaguti (1971, 1963b, 1958). Keys to the Trematoda Vols. 1-3 (Gibson *et al*., 2002, Jones *et al*., 2005, Bray *et al*., 2008) were referred.

Obervations were recorded to calculate the prevalence and intensity of helminth infection in the fish hosts.

2.3. Analysis of Prevalence data: Data were recorded on the prevalence and intensity of helminth infection in piscine hosts and the data were analyzed following Bush *et al*., (1997):

Prevalence (P) = the number of infected hosts with one or more individuals of a particular parasites species (or taxonomic group) divided by the number of hosts examined.

Mean intensity (MI) = the average intensity, i.e., the total number of parasites of a particular species found in a sample divided by the number of hosts infected.

Abundance (A) = the total number of individuals of a particular parasite species in a sample of a particular host species divided by the total number of hosts (including both infected and uninfected) of that species examined.

2.4. Molecular characterization

DNA isolation & PCR Amplification

The genomic DNA was extracted from the alcohol fixed sample by using phenol chloroform method of Sambrook et al., (1989). For PCR-amplification we used two rDNA marker gene regions: 28S and internal transcribed spacer 2 (ITS2). Primers used for the respective gene region are detailed as follows: NC5 (forward)/ NC2 (reverse) (Zhu et al. 1999) for ITS2 and 39KF (forward) and 501R (reverse) for 28S. The PCR conditions are performed as follows: initial denaturation at 94 ºC (5min), annealing – for 28S at 57 ºC (1 min), ITS at 57 ºC (1.10 min) and final extension at 72 ºC (10 min). The amplified PCR products were separated by electrophoresis through 1.5 % (w/v) agarose gels in TAE buffer, stained with Sybr safe gel stain, transilluminated under ultraviolet light, and then photographed. PCR products were sent for sequencing in both directions at Agrigenome, Kochi, Kerala, India.

Analysis of the sequences

All the analyses were done using MEGA7 (Kumar et al. 2016). The sequences were edited using the software package, FinchTV 1.4.0 (Geospiza, Inc.; Seattle, WA, USA; http://www.geospiza.com), and alignment was performed using Clustal X 1.64 (Thompson et al., 1997). Genetic distance was calculated by pairwise comparisons of sequences using the Kimura 2-Parameter method under Gamma distribution. The phylogenetic relationship was generated using the maximum likelihood (ML) and the with all the available representative species of the genus *Prosorhynchoides* retrieved from GenBank. The ML tree was constructed using K2PG (Kimura 2 parameter? Gamma distribution) substitute on models with 1000 bootstraps for ITS and 28S rDNA dataset respectively.

2.5. Scanning Electron Microscopy (SEM)

The collected specimens were washed thoroughly in 0.7% saline solution and fixed in 10% cold neutral buffered formalin (NBF) at 4^0C for 12-18 h. Following fixation, the specimens were washed in phosphate buffer and dehydrated with ascending grades of alcohol. In lieu of critical-point drying, the specimens were dehydrated in ascending grades of acetone, treated with tetramethyl silane [TMS-$(CCH3)_4$Si, boiling point 26.3ºC, surface tension 10.3 dynes/cm at 20ºC] for 10 minutes and TMS was dried off at 25º C (Roy and Tandon, 1991). The dried samples were metal coated with gold in a fine coat ion sputter JFC-1100(JEOL). Observations were made with the scanning electron microscope JSM 6360(JEOL) and LEO 435 VP SEM at electron-accelerating voltages ranging between 10-20 kV.

3 KEY FINDINGS AND RESULTS

- Fishes were collected from different rivers of Mizoram. Emphasis was given to those fish species, which are commonly used in local traditional cuisine in selected pockets of Mizoram.
- 20 different species of piscine host were collected and examined. Some of the pictures are given below.

Fig2: *Mastacembelus armatus*

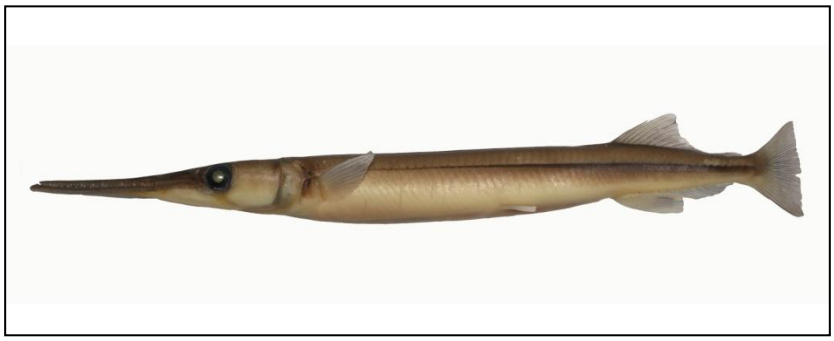


Fig3: *Xenentodon cancila*

Fig4: *Glyptothorax maceriatus*

Fig5: *Channa punctata*

Fig6: *Schistura nebeshwari*

Fig7: *Pseuoluguvia spicula*

Fig.8: *Tor tor*

3.1 Prevalence Status:

154 *M. armatus* were collected from different river sites in which Prevalence, Mean intensity and Abundance was found to be highest among the fishes collected from Tuikum

Locality	No of host examined	Total No of parasites	Prevalence (%)	Mean Intensity	Abundance
Chhimtuipui	15	10	66.7	10	6.67
Sairang	29	15	51	2.13	1.10
Tuichang	10	6	60	1.67	1
Tuikum	2	2	100	18	18
Tuirial	98	70	71	2.18	1.56

Table1: Diversity of helminth parasites in *Mastacembelus armatus* from different collection sites

40 *X. cancila* were collected from different river sites in which Prevalence, mean intensity and Abundance was again found to be highest in the fishes collected from Tuikum.

Locality	No of host examined	Total No of parasites	Prevalence (%)	Mean Intensity	Abundance
Tuirial	15	58	73.34	5.28	3.86
Tuichang	9	17	66.67	2.84	1.89
Tuikum	5	73	80	18.25	14.6
Chhimtuipui	2	-	-	-	-
Sairang	5	-	-	-	-
Muthi	4	6	50	3	1.5

Table2: Diversity of helminth parasites in *Xenentodon cancila* from different collection sites

60 *G. maceriatus* were collected from 5 river sites, Prevalence were found to be highest in fishes collected from Muthi river whereas Mean intensity and Abundance were found to be highest in fishes collected from Sekulh.

Collection sites	No of host collected	No of host infected	Prevalence (%)	Mean Intensity	Abundance
Muthi	6	4	66.7	5.5	3.67
Sairang	16	10	62.5	4.1	2.56
Sekulh	5	3	60	8	4.8
Tuivai	5	3	60	6.67	4
Tuirial	28	16	57.15	1.37	0.78

Table3: Diversity of helminth parasites in *Glyptothorax maceriatus* from different collection sites.

35 *Channa* spp were collected from different river sites in which Prevalence, Mean intensity and Abundance was found to be highest among the fishes collected from Sairang.

Collection sites	No of host collected	No of host infected	Prevalence (%)	Mean Intensity	Abundance
Tuirial	10	3	30	0.5	0.6
Sairang	2	2	100	4	4
Kolasib	10	1	10	1	0.1
Tuikum	3	1	33.34	1	0.34
Tuichang	6	3	50	2	1
Lau	4	2	50	1.5	0.75

Table4: Diversity of helminth parasites in *Channa* spp from different collection sites

36 *Schistura pausireticulata* were collected from different Sairang and Tuirial river sites in which Prevalence and Abundance was found to be higher among the fishes collected from Sairang whereas mean intensity was higher in fishes collected from Tuirial.

Collection sites	No of host collected	No of host infected	Prevalence (%)	Mean Intensity	Abundance
Tuirial	33	5	15.15	3	0.45
Sairang	3	1	33.34	0.34	1

Table5: Diversity of helminth parasites in *Schistura pausireticulata* from different collection sites

17 *Pseudoluguvia spicula* were collected from Sairang river sites in which Prevalence, Mean intensity and Abundance were given below

Collection sites	No of host examined	No of host infected	Prevalence (%)	Mean Intensity	Abundance
Sairang	17	10	58.82	24	14.11

Table6: Diversity of helminth parasites in *Pseudoluguvia spicula* from different collection sites

26 *Tor tor* were collected from different river sites in which Prevalence was highest in in fishes collected from Tuivai river, Mean intensity and Abundance was found to be highest among the fishes collected from Mar river.

Locality	No of host examined	No of host infected	Prevalence (%)	Mean Intensity	Abundance
Muthi	5	-	-	-	-
Tuivai	3	3	100	6.7	4
Mar	10	6	60	18.25	14.6
Kawlchaw	3	2	67	10	6.7
Tlawng	5	2	40	3	1.2

Table 6: Diversity of helminth parasites in *Tor tor* from different collection sites

Locality	No of host examined	No of host infected	Prevalence (%)	Mean Intensity	Abundance
Muthi	**5**	**-**	**-**	**-**	**-**
Tuivai	**3**	**3**	**100**	**6.7**	**4**
Mar	**10**	**6**	**60**	**18.25**	**14.6**
Kawlchaw	**3**	**2**	**67**	**10**	**6.7**
Tlawng	**5**	**2**	**40**	**3**	**1.2**

Table 7: Diversity of helminth parasites in different fish Hosts

Name of hostfq3esdcrcv `	No of host examined	No of host infected	Prevalence (%)	Mean Intensity	Abundance
Mastacembelus armatus	**193**	**185**	**95.85**	**4.18**	**4.01**
Xenentodon cancila	**40**	**23**	**57.5**	**6.69**	**3.85**
Glyptothorax maceriatus	**85**	**79**	**92.94**	**2.03**	**1.89**
Channa marulius	**35**	**12**	**34.28**	**2.08**	**0.71**
Berilius bendelensis	**45**	**6**	**13.34**	**1.17**	**0.15**
Garra manipurensis	**36**	**2**	**5.56**	**1.5**	**0.08**
Devario aequipinnatus	**39**	**12**	**30.76**	**1.67**	**0.15**
Pseudocheneis koladyne	**1**	**1**	**100**	**11**	**11**
Clarias magur	**2**	**2**	**100**	**11**	**11**
Puntius sophore	**1**	**1**	**100**	**1**	**1**
Glossogobius giuris	**6**	**4**	**66.67**	**5**	**3.34**
Badis badis	**42**	**2**	**4.76**	**1.5**	**0.07**
Paracanthocobiti	**21**	**3**	**14.28**	**3**	**0.42**

s botia					
Cirrhina reba	**1**	**-**	**-**	**-**	**-**
Psilorhynchus gracilis	**5**	**-**	**-**	**-**	**-**
Tilapia mossambica	**1**	**-**	**-**	**-**	**-**
Pethia chonchonius	**34**	**-**	**-**	**-**	**-**
Pseudoluguvia spicula	**17**	**10**	**58.82**	**24**	**14.11**
Schistura pausireticulata	**36**	**6**	**16.67**	**2.67**	**0.48**
Tor tor	**26**	**13**	**50**	**5.76**	**2.88**

Table 8: Diversity of helminth parasites in different fish Hosts

Prevalence of parasites were found to be highest in *Pseudocheneis koladyne*, *Clarias magur* and *Puntius sophore*. Mean intensity and abundance was found to be highest in *Pseudoluguvia spicula*. Whereas, no parasites were recovered from *Cirrhina reba*, *Psilorhynchus gracilis, Tilapia mossambica* and *Pethia chonchonius*.

There is lot of variation in the prevalence of parasite among fishes collected from different river sites, but it might be interesting to note that the river Tuirial which flows near the human settlement, and also near the city dumping ground, tends to harbour more diverse group of parasites. With mostly all the three group of parasites present in a single host. This is in agreement with Moller and Anders (1986) who concluded that fish from more polluted water tend to harbour more helminth parasites than those from less polluted waters. As was observed in the present studies the prevalence, intensity and abundance also varied with different rivers from which the fish was collected. Polanski (1961) summarised that the main factors determining the variety of parasite fauna as well as the intensity and incidence of infection can depends on the diet of the host, lifespan of the host, the mobility of the host throughout its life including the variety of habitats it encounters, its population density and the size attained, large hosts provide more habitats suitable for parasites than do small ones. During this study, we find that carnivorous fish such as *M. armatus*, *X. cancila* and *Channa maurulius* were the most heavily infected of all the fish that we have collected due to their voracious feeding habit and forages at night on benthic insect larvae, worms and some submerged plant material thus making them more susceptible to helminth infections. The feeding pattern of fish is an important factor in their infestation with parasites. Luque and Poulin (2004) reported that predatory fish species harbor a greater diversity and abundance of larval helminths than herbivorous and planktivorous species. Predatory fish are exposed to more infective helminth larvae in their diet; thereby making them more susceptible to higher parasite colonization than phytophagous and planktivorous fish.

The helminth communities of these freshwater fishes are species-poor, and that, considerable proportion of fish from the region is uninfected or lightly infected.

3.2 Morphological analysis:

- Cestodes were found to infect *M. Armatus* and *Channa* spp
- The pseudophyllidean cestodes recovered belongs to the genus *Senga* sp.

1. Cestoda

Family: Bothriocephalidae Blanchard, 1849
Genus: *Senga* Dollfus, 1934
Host: *M.armatus* and *Channa* spp.

Description:

Scolex pear shaped; bothria shallow; immature proglottids longer than wide; mature and gravid proglottids wider than long; genital pore dorsal, virtually median; testes medullary, in two lateral fields; ovary bilobed, median posterior region of proglottid, eggs nonoperculate, unembryonated.

Fig 9: *Senga* sp.

- An Acanthocephalan species belonging to the genus *Neoechinorhynchus* sp. were recovered from *Xenentodon cancila*.

2. Acanthocephala

Family: Neoechinorhynchidae Van Cleave, 1919

Genus : *Neoechinorhynchus* Hamann, 1892

Host: *Xenentodon cancila*

Description:

Acanthocephala was found in the fishes collected from Tuichang river only. Male body was 5.6 mm long and the broadest region measured 0.5 mm. Trunk aspinose, small, cylindrical bowed or straight, proboscis short somewhat globular, proboscis receptacle subcylindrical somewhat short, lemnisci digitiform to filiform, lemnisci twice as long as proboscis sheath, testis in posterior half of the trunk, testis oval,cement gland syncytial and cement reservoir rounded, everted bursa, proboscis receptacle short, simple, single-walled sac. Lemnisci slender elongate, unequal.

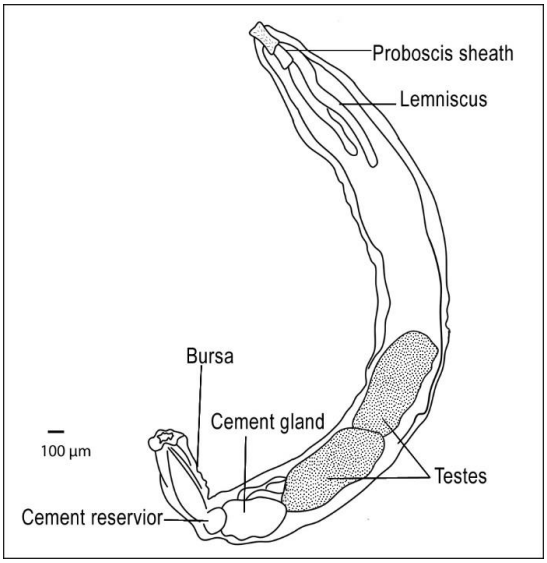


Fig10: *Neoechinorhynchus*

- The Trematodes collected were identified as Digenean belonging to the genus *Prosorhynchoides aspinosus* n.sp., *Plagioporus* sp. and *Phyllodistomum* sp. *Bucephalus* and *Podocotyloides* sp. And were recovered from *Glyptothorax maceriatus*, *M. Armatus* and *Xenentodon cancila* and *Pseudoluguvia spicula.*

3. Trematoda

1. Family: Bucephalidae Poche, 1907

Genus: *Prosorhynchoides* Dollfus, 1929

Host: *Xenentodon cancila*

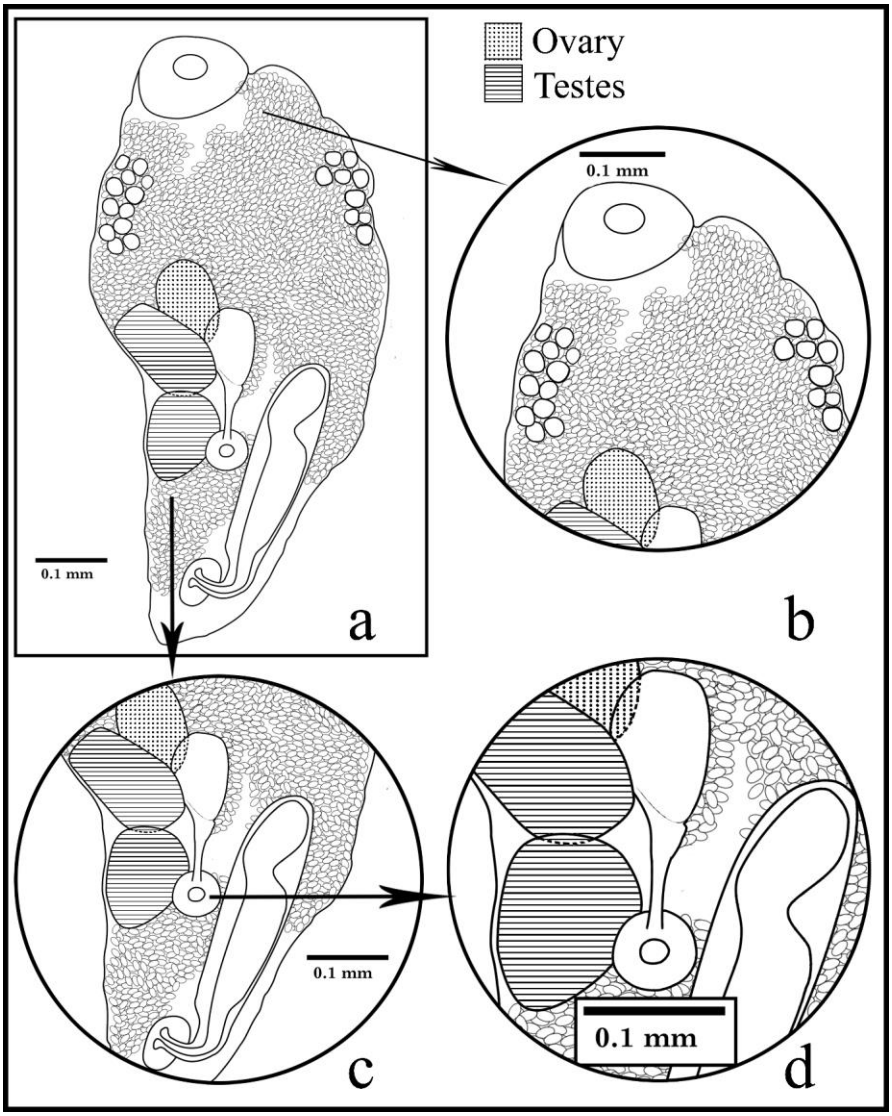
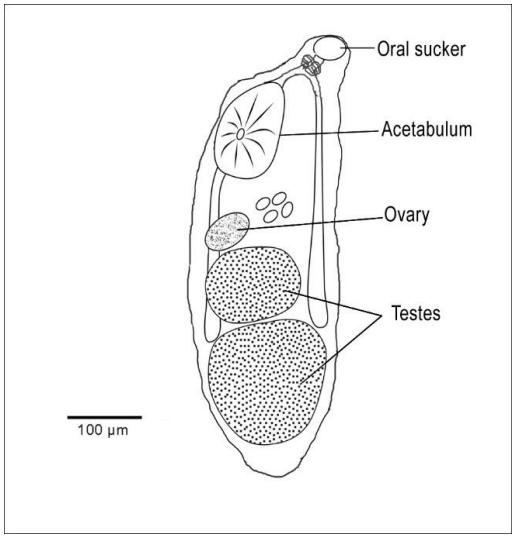


Fig11: *Prosorhynchoides* sp. a. whole mount (ventral view); b. Enlarged view showing rhynchus and vitelline follicle; c. Enlarged view showing cirrus sac, testes and ovary; d. Enlarged view showing pharynx and sac-like caecum.

Description:

Body small, inversely pear-shaped, broader at the anterior region than posterior. Body measured 0.168-1.10 mm in length and maximum width was 0.441-0.576 mm at post oral region. Rhynchus (anterior sucker) was large. Digestive cecum globular sac like, usually lies at level of anterior testis. Ovary pretesticular, testes somewhat oval, cirrus sac cylindrical, extended from level of anterior or posterior testis to slightly posterior end of the body. Genital pore terminal at posterior extremity.

Vitellaria compact, in 2 groups each with 14–15 rounded follicles arranged in lateral body spaces, anterior extent of vitellaria upto rhynchus.



2. Family:Opecoelidae Ozaki, 1925

Genus :*Plagioporus* Stafford,1904

Host: *Xenentodon cancila*

Fig 12: *Plagioporus* sp.

Description:

Body elongate measuring 0.8–0.97 x 0.24–0.25 mm, aspinose, forebody distinctly tapered, oral sucker terminal, oral sucker visibly smaller than acetabulum 0.15 x 0.14 mm which is located in anterior of the body, testes tandem in posterior third of the body anterior testis 0.11 x 0.13 mm, posterior testis 0.14 x 0.15 mm eggs large measuring 0.06 x 0.02 mm and elliptical in shape, Ovary ovoid just anterior to anterior testis, pharynx located behind the anterior sucker, Vitellaria extending to the anterior region near the acetabulum.

3. Family: Gorgoderidae Looss, 1899

Genus: *Phyllodistomum* Braun, 1899

Host: *Xenentodon cancila, M.armatus* and *Channa* spp

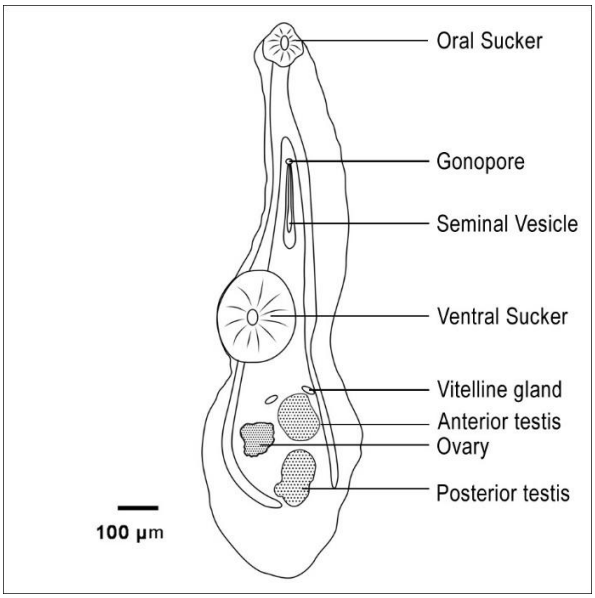


Fig 13: *Phyllodistomum* sp.

Description:

Body dorsoventrally flattened, slightly spatulate, divided into a narrow tubular, curved forebody and posterior part of the body extended into bolbous shape. Measuring 1.48 mm in length and 0.45 mm in width across the widest portion. Oral sucker, terminal, slightly oval measuring 0.18 × 0.15 mm, mouth opening ventrally, no noticeable papillae on oral sucker. Ventral sucker measuring 0.27×0.23 mm was larger than oral sucker.

Muscular pharynx present. Ovary oval parallel to anterior testis, post-equatorial, testes two in number located in the broadest part of hind body, post-equatorial, tandem, inter-caecal and lobed.

4. Family: Bucephalidae Poche, 1907

Genus: *Bucephalus* Baer, 1826

Host: *Sperata*

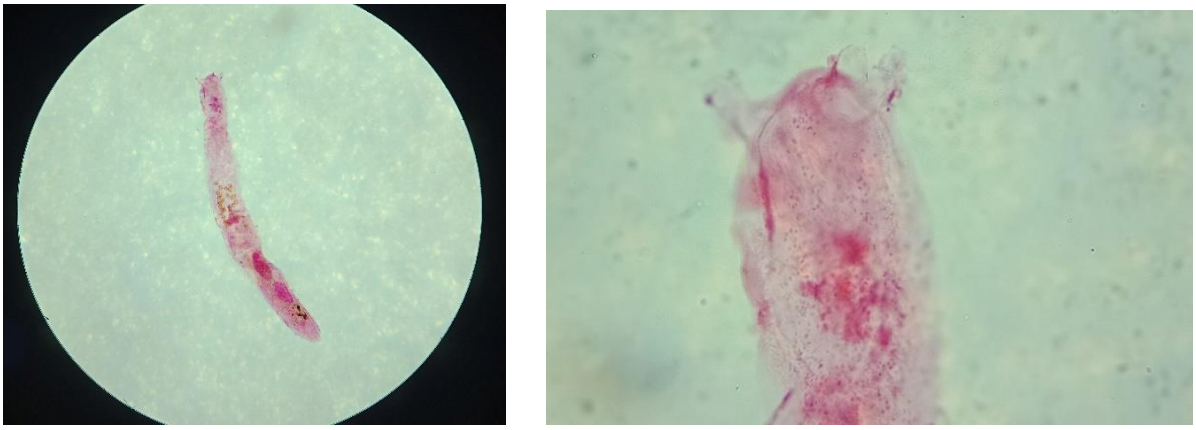


Fig 14: *Bucephalus* sp. Showing whole worm and anterior end with tentacles.

Description: Anterior rhynchus a simple sucker, usually surrounded with seven tentacles around antero dorsal rim of sucker, caecum saclike and not bifurcated, testes oblique, seminal vesicle spherical to ovoid, ovary pretesticular.

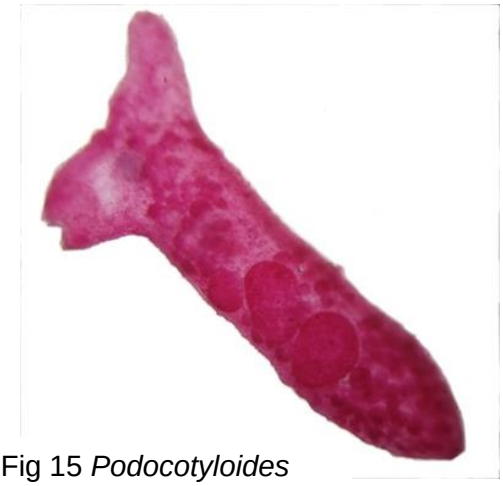


Fig 15 *Podocotyloides*

5. Family: Opecoelidae

Genus: *Podocotyloides,* Yamaguti,1934

Host: *Sperata*

.

Description: Oral sucker terminal or sub terminal, ventral sucker on muscular peduncle, testes 2 tandem, cirrus sac short, ovary near mid body, pretesticular.

• The nematodes recovered belong to the genus *Spinitectus* sp., *Paracamallanus* sp., and *Capillaria* sp.

Fig 16 *Spinitectus* sp.

4. Nematoda

1. Family: Cystidicolidae Skrjabin, 1946
Genus: *Spinitectus* Fourment, 1884
Host: *Xenentodon cancila,M.armatus* and *Channa* spp

Description:
Small, whitish in colour, surface of the body covered with transverse ring of spines.

2. Family: Camallanidae Railliet and Henry, 1915
Genus: *Paracamallanus* Yorke and Maplestone, 1926
Host: *M.armatus* and *Channa* spp

Fig17: *Paracamallanus* sp.

Description:
Mouth slit-like; buccal capsule consisting of two lateral chitinous valves, with longitudinal rib-like thickenings internally, trident present; basal chitinous ring between valves and oesophagus

present, long posterior glandular part, vulva about middle of body; uteri opposed; position of anal opening 0.18 from posterior end.

8. Family: Capillaridae

Genus: *Capillaria* Zeder, 1800

Host: *Sperata*

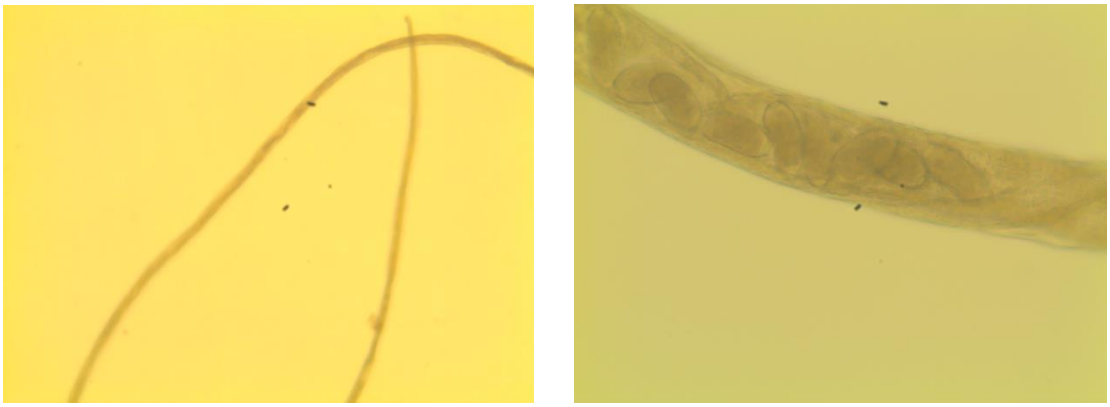


Fig 18: *Capillaria* sp. Showing anterior end and eggs in female.

Description: Small, filiform nematode, Anterior end of body narrow, rounded. Lateral bacillary bands extending along almost whole-body length, muscular oesophagus long, narrow. Eggs with bipolar plugs.

- Trematodes were found to be the most common helminth parasites infecting the four different fish host followed by nematodes which infect three different hosts whereas cestodes and Acanthocephala infected two different hosts.
- Of the fish host examined for recovery of the helminth *M. armatus* is the only fish where we find multiple infections with nematode, cestode and trematode.

3.3. Molecular analysis:

- The total length of ITS-2 rDNA and 28S rDNA sequences obtained are 363bp and 971bp respectively.
- The ITS-2 rDNA and 28S rDNA sequences of the present species were compared with all the sequence data of bucephalids available in NCBI GenBank. The 3 developed ITS-2 rDNA sequences did not show any variation.
- *Prosorhynchoides aspinosus* ITS-2 sequences closely related with *P. karvei* (95.08% similarity with 4% K2P distance), *P. galaktionovi* (94.26% similarity with 4.5% K2P distance), *P. cutmorei* (94.26% similarity with 4.5% K2P distance), *P. moretonensis* (93.24% similarity with 6.7% K2P distance), *P. kohnae* (92.90% similarity with 6.1% K2P distance) *P. waeschenbachae* (92.60% similarity with 6.3% K2P distance) while all other *Prosorhynchoides* species available in the GenBank showed less than 90% similarity.
- Simultaneously, analysis of 28S rDNA sequence with other *Prosorhynchoides* spp. from GenBank showed that the new species is closely related to *P. kohnae* (99.08% similarity with 8 bp difference) followed by *P. galaktionovi* (98.87%), *P. cutmorei* (98.53% with 14 bp difference), *P. waeschenbachae* (98.25% with 16 bp difference) and *P. moretonensis* (97.84% with 20 bp

difference), *P. paralichthydis* (95.58% with 43 bp difference), *P. gracilescens* (95.07% with 48 bp difference) whereas all the other *Prosorhynchoides* spp available in the GenBank showed less than 95% similarity.

3.4. Phylogeny:

- The maximum likelihood (ML) tree of both ITS-2 rDNA and 28S rDNA datasets exhibited almost similar topology with strong bootstrap support. The phylogeny using the two gene datasets revealed that *Prosorhynchoides* parasitizing belonid fishes were grouped and *P. aspinosus* n. sp. forms a strongly supported clade (98% and 86%) with other species of *Prosorhynchoides* from bonid fishes, viz. *P. karvei, P. cutmorei, P. kohnae, P. galaktionovi, P. moretonensis, P. waeshenbachae*, *P. galaktionovi* and *P. kohnae*. Similarly, those parasitizing sciaenid fish, viz. *P. caecorum* and *P. megacirrus* also cohesively grouped with strong bootstrap support (91% and 96%).
- *Phyllodistomum* sp. from Mizoram form a clade with *Phyllodistomum parorchium* and *Pseudophyllodistomum srivastavai* which are also reported from freshwater fishes in India with a strong support of 100% bootstrap value.

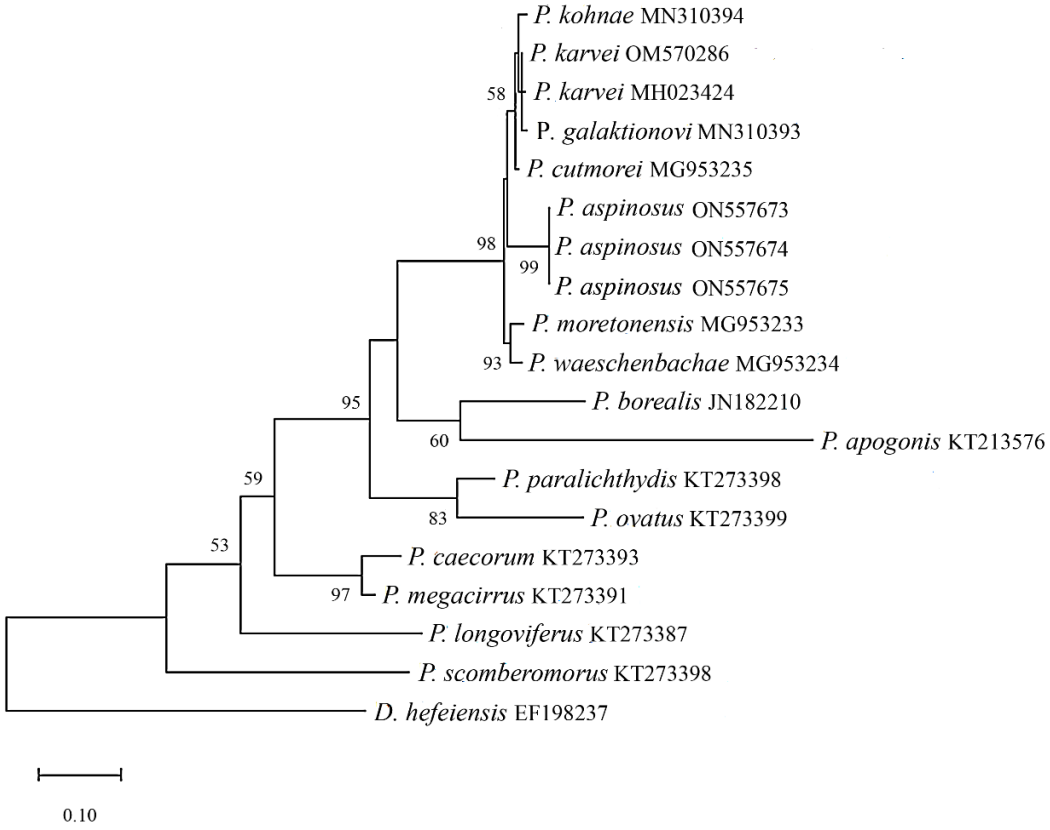


Fig 19 (a): Maximum Likelihood (ML) tree based on ITS-2 rDNA sequences showing the relationships of *Prosorhynchoides* spp.

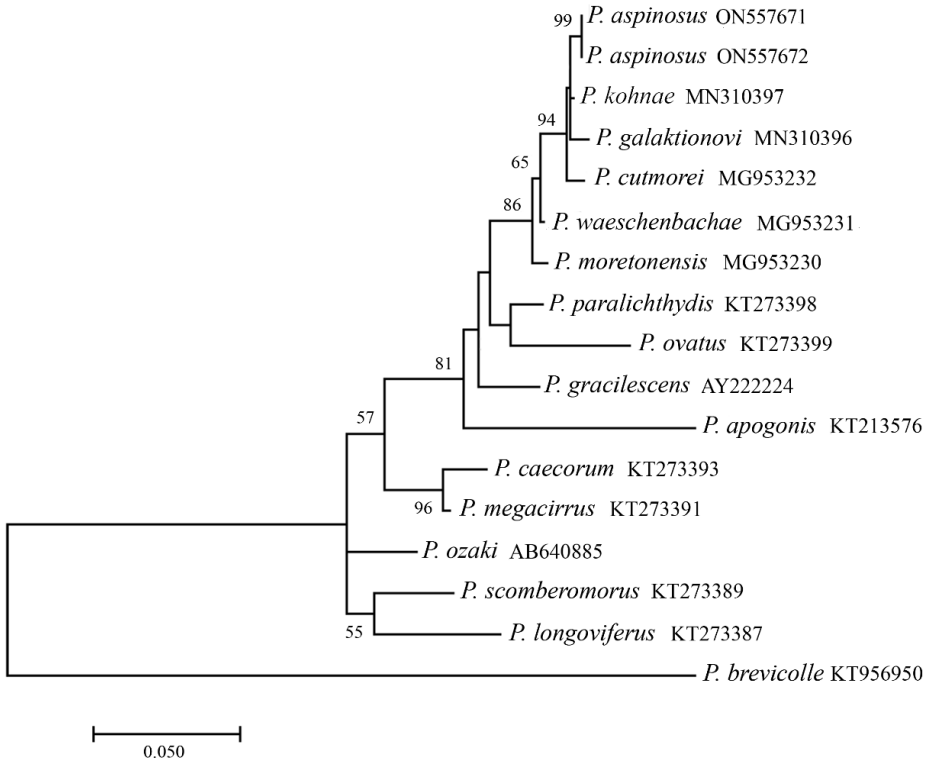


Fig 19 (b): Maximum Likelihood (ML) tree based on 28S rDNA sequences showing the relationships of *Prosorhynchoides* spp.

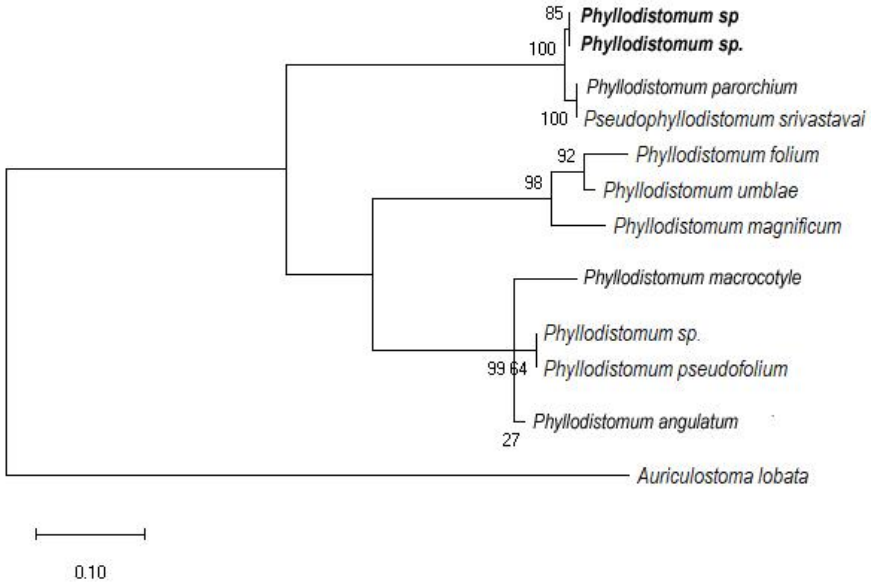


Fig 20: Phylogenetic relationship of *Phyllodistomum* sp. with other *Phyllodistomum* spp. based on analysis of the ITS rRNA region.

3.5. Scanning Electron Microscopy:

The SEM studies revealed detailed surface topography of the fluke with several characteristic features (Fig. 2). The picture shows the body as having a rounded anterior with a terminal rhynchus, having maximum width in the mid-region and tapering posteriorly with an almost pointed end clearly showing the excretory and genital pores. Studies on surface fine topography further reveal the presence of distinct knob-like protrusions on the tegument which were not visible under a light microscope. Each protrusion is without any cutting plate or spine, and at higher magnification, appears to be granulated. The arrangement of these protrusions is irregular all over the tegument such that they seem to form a group. These groups of knob-like protrusions were separated by horizontal grooves. No knob-like protrusion is present in the posterior end region of the body which, however, showed the arrangement of longitudinal ridges. No papillae or spines are observed in the area near the excretory and vaginal pores.

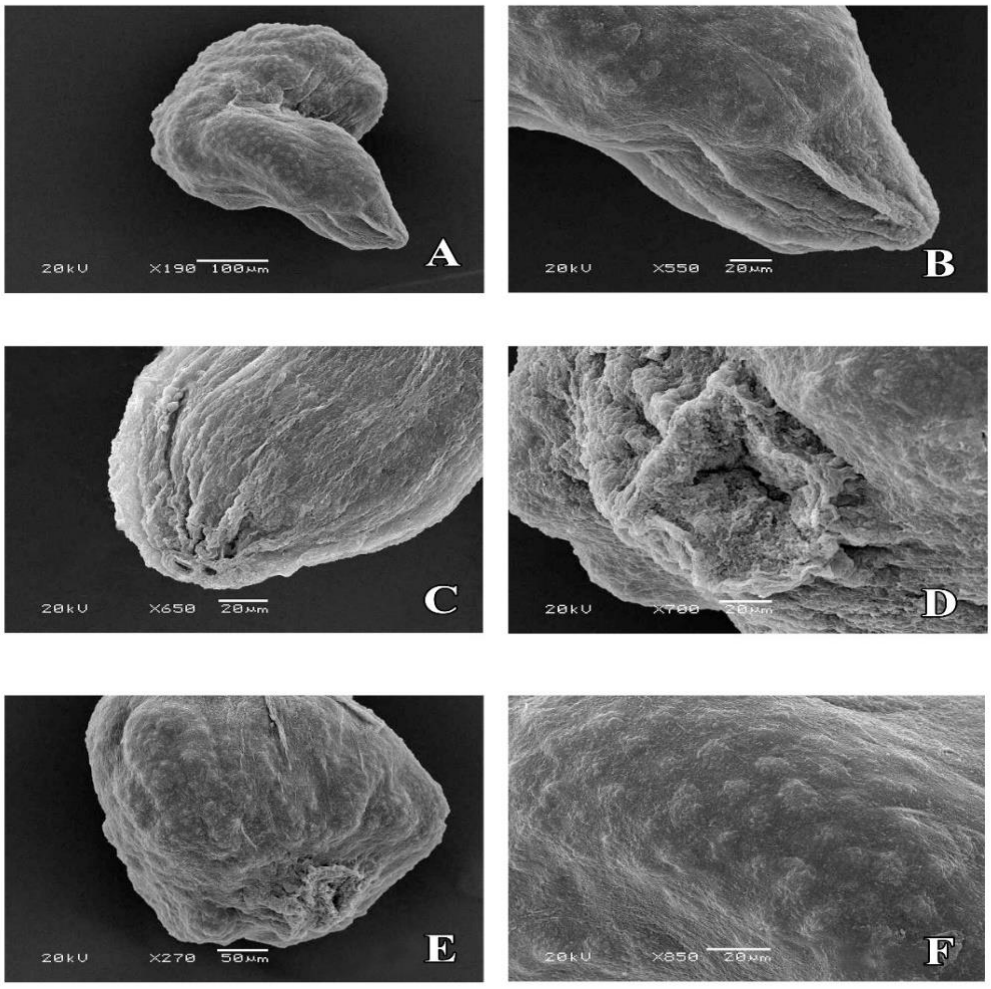


Fig 21: SEM picture of *Prosorhynchoides aspinosus* n. sp. (A) Whole worm (B) Posterior end (C) Excretory and vaginal pore (D) Rhynchus (E) Anterior end showing rhynchus (F) Tegumental elevation.

Table 9: Physico chemical analysis of water in Mizoram: (WATER QUALITY DATA OF RIVERS MONITORED UNDER NATIONAL WATER QUALITY MONITORING PROGRAMME (NWMP), 2021**)**

STATION CODE	NAME OF MONITORING LOCATION	TEMPERATURE (°C)		DISSOLVED OXYGEN (mg/L)		pH		CONDUCTIVITY (µmhos/cm)		BIO- CHEMICAL OXYGEN DEMAND (mg/L)		NITRATE (mg/ L)	
		MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX
PRIMARY WATER QUALITY CRITERIA NOTIFIED UNDER E(P) RULES, 1986				**> 5.0**		**6.5-8.5**				**< 3.0**			
1	RIVER TLAWNG UPSTREAM AIZAWL	18.0	27.0	4.5	8.6	7.3	8.4	102	378	1.0	2.0	0.30	0.10
2	RIVER TLAWNG DOWNSTREAM AIZAWL	18.0	27.0	4.3	8.1	6.7	8.3	98	1113	1.0	2.3	0.30	0.12
3	RIVER TUIRIAL UPPER CATCHMENT	18.0	29.0	5.1	7.7	7.2	8.2	102	291	1.0	2.1	0.30	0.30
4	RIVER TUIRIAL LOWER CATCHMENT	18.0	29.0	5.5	7.6	7.2	8.5	132	274	1.0	2.6	0.30	0.30
5	RIVER TLAWNG SAIRANG	19.0	30.0	5.0	11.5	6.1	8.6	117	282	1.0	2.4	0.30	0.30
6	RIVER TUIRIAL RIVER, AIRFIELD, NR DUMPING GROUND	19.0	29.0	4.5	7.9	7.3	9.4	101	271	1.0	2.2	0.30	25.20
7	RIVER DAMDIAI STREAM, AIRFIELD AFTER DUMPING GROUND	18.0	29.0	5.0	7.3	7.2	8.2	93	225	1.0	2.3	0.30	0.30
8	RIVER TUIRIAL RIVER, AIRFIELD AFTER DUMPING GROUND	18.0	30.0	4.4	7.6	7.4	9.6	100	266	1.0	1.9	0.32	0.05
9	RIVER CHITHE STREAM ARMED VENG NR MINI SPORTS COMPLEX	18.0	30.0	3.3	7.2	5.9	8.0	133	812	1.0	5.9	0.35	1.05
10	RIVER TUIRINI RIVER, SELING	12.0	22.0	7.0	11.9	6.5	7.8	27	279	1.0	1.7	0.30	0.04

11	RIVER TUIVAWL NEAR TUIVAWL BRIDGE, KEIFANG	12.0	21.0	6.7	12.0	6.2	8.2	66	284	1.0	1.7	0.30	0.05
12	RIVER MAT RIVER	17.0	27.0	6.6	7.4	5.8	8.1	34	191	1.0	1.6	0.30	0.03
13	RIVER CHHIMTUIPUI RIVER, KAWLCHAW	18.0	23.0	7.2	8.7	6.8	7.8	85	284	1.0	2.1	0.30	0.22
14	RIVER TUIKUM RIVER, SERCHHIP	14.0	21.0	6.3	7.9	7.1	8.1	52	224	1.0	1.7	0.30	0.05
15	RIVER TUT RIVER, NEAR DAPCHHUAH VILLAGE	21.0	30.0	5.9	10.0	6.2	7.8	88	200	1.0	1.9	0.30	0.06
16	RIVER TUIPUI DARZOKAI RIVER, CHHIMTUIPUI, TUIPUID VILLAGE	16.0	26.0	6.5	8.5	6.4	8.2	44	549	1.0	1.7	0.30	0.03
17	RIVER TUIPUI, U/S NR CONFLUENCE PT KEILUNGLIAH STREAM, VENGTHAR, CHAMPHAI	8.0	22.0	3.3	7.2	6.4	8.7	115	549	1.0	1.8	0.30	0.11

4 OVERALL ACHIEVEMENTS

- In the state of Mizoram Northeast India, a biodiversity hotspot, fishes constitute an important part of the diet and through recent survey prevalence of fish borne helminth infections have been reported especially from rural areas but there is no published record of them to date.
- Fishes were collected from the different riverine systems of Mizoram covering different districts.
- Twenty different species of fishes were examined for recovery of the parasite,
- The recovered helminth parasites include nematode, cestodes, trematodes and an acanthocephala, in which trematode was found to be the most prevalent and abundant group whereas acanthocephala was the least encountered.
- The pseudophyllidean cestodes recovered belongs to the genus *Senga* sp.
- An Acanthocephalan species belonging to the genus *Neoechinorhynchus* sp.
- The Trematodes collected were identified as Digenean belonging to the genus *Prosorhynchoides aspinosus* n.sp., *Plagioporus* sp. and *Phyllodistomum* sp. *Bucephalus* and *Podocotyloides* sp. And were recovered from *Glyptothorax maceriatus*, *M. Armatus* and *Xenentodon cancila* and *Pseudoluguvia spicula.*
- The nematodes recovered belong to the genus *Spinitectus* sp., *Paracamallanus* sp., and *Capillaria* sp.
- Among the fishes *Mastacembelus armatus* belonging to the family Mastacembelidae is the only fish which harbour three different groups of the helminth parasites.

- Prevalence of parasites were found to be highest in *Pseudocheneis koladyne*, *Clarias magur* and *Puntius sophore*. Mean intensity and abundance was found to be highest in *Pseudoluguvia spicula*. Whereas, no parasites were recovered from *Cirrhina reba*, *Psilorhynchus gracilis, Tilapia mossambica* and *Pethia chonchonius*.
- There is lot of variation in the prevalence of parasite among fishes collected from different river sites, but it might be interesting to note that the river Tuirial which flows near the human settlement, and also near the city dumping ground, tends to harbour more diverse group of parasites. With mostly all the three group of parasites present in a single host.

- Trematodes were found to be the most common helminth parasites infecting the four different fish host followed by nematodes which infect three different hosts whereas cestodes and Acanthocephala infected two different hosts.
- ITS2 and 28S rDNA sequence generated for *Prosorhynchoides* sp. does not show high similarities with any other sequences obtained from species of *Prosorhynchoides* from GenBank.
- *Phyllodistomum* sp. from Mizoram form a clade with *Phyllodistomum parorchium* and *Pseudophyllodistomum srivastavai* which are also reported from freshwater fishes in India with a strong support of 100% bootstrap value.

- SEM Studies on surface topography of *Prosorhynchoides aspinosus* n.sp. further reveal the presence of distinct knob-like protrusions on the tegument which were not visible under a light microscope. Each protrusion is without any cutting plate or spine, and at higher magnification, appears to be granulated.
- Besides fishes, helminth parasites such as metacercaria of some digenea and Aspidogastrea were recovered from Crab and snails from the river of Mizoram, which are yet to be identified.

5 IMPACTS OF FELLOWSHIP IN IHR

5.1 Socio-Economic Development (max. 500 words, in bullet points)
5.2 Scientific Management of Natural Resources In IHR (max. 500 words, in bullet points)
5.3 Conservation of Biodiversity in IHR (max. 500 words, in bullet points)
5.4 Protection of Environment (max. 500 words, in bullet points)
5.5 Developing Mountain Infrastructures (max. 500 words, in bullet points)
5.6 Strengthening Networking in IHR (max. 700 words, in bullet points)

6 EXIT STRATEGY AND SUSTAINABILITY

- During the survey besides the helminth parasites collected from freshwater fishes we have encountered many parasites from freshwater crab and snails such as digenea,

- Aspidogastrea and certain type of cercaria which further need to be thoroughly analysed and identified and larger area still need to be covered as many areas could not be covered for collection of specimens due to covid restrictions.

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Sequences submitted:

***Prosorhynchoides aspinosus*.sp. ITS2 – Accession No- ON557673, ON557674, ON557675**
28S – Accession No- ON557671, ON557672

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9 APPENDICES

1. Chenkual Malsawmtluangi, Lalramliana, A new species of Prosorhynchoides Dollfus, 1929 (Digenea: Bucephalidae) from Xenentodon cancila Hamilton, 1822 in Mizoram, Northeast India, Parasitology International, Volume 92, 2023, 102690, ISSN 1383-5769, https://doi.org/10.1016/j.parint.2022.102690.
2. Malsawmtluangi, C. and Lalramliana: Studies on the distribution and diversity of helminth infection in *Xenentodon cancila* (Hamilton, 1822) in Mizoram, Northeast India. J. Environ. Biol., 41, 832-839 (2020). http://doi.org/10.22438/jeb/4(SI)/MS_1901
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   (Received best poster Award: On the topic **'Studies on the distribution and diversity of helminth infection in *Xenentodon cancila* from different rivers of Mizoram, Northeast India'.)**

(Signature of HRA)

(NMHS FELLOWSHIP COORDINATOR)

(HEAD OF THE INSTITUTION)

Principal
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Place: Aizawl
Date: 28/11/2022

PACHHUNGA UNIVERSITY COLLEGE AIZAWL

PART B: COMPREHENSIVE REPORT

(HJRF)

Title: Diversity and sustainable utilization of the entomopathogenic bacteria (*Xenorhabdus* and *Photorhabdus*) from Mizoram, northeast India

1 INTRODUCTION

Photorhabdus and *Xenorhabdus* are gram negative bacteria belonging to a family Enterobacteriaceae which are symbiotically associated with entomopathogenic nematodes (EPNs) viz *Heterorhabditis* and *Steinernema*, respectively (Boemare, 1993). After gaining entry to the insect larvae, the infective juvenile (IJs) releases their symbiotic bacteria into the hemocoel where the bacteria multiply and release a number of virulence factors that kills the insect hosts within 24–48 hrs by septicemia. The bacteria proliferate inside the dead insect while the nematodes in turn feeds on the bacteria and remnants of the insect tissues for growth and reproduction (Boemare, 2002).

Several compounds produced by these bacterial symbionts are known to protect insect cadaver micro environment due to their antimicrobial, nematicidal and insecticidal activity. When the mutualistic association infects the insect larvae, several compounds produced by these bacterial symbionts are known to protect insect cadaver micro environment due to their antimicrobial, nematicidal and insecticidal activity (Muangpat et al., 2017, 2020) (Chen et al., 1994) (Hu et al., 2006). The bacteria proliferate inside the dead insect while the nematodes in turn feeds on the bacteria and remnants of the insect tissues for growth and reproduction (Boemare, 2002). When the food resources are depleted, the IJs emerge from the host cadaver to search for a new host (Wang and Gaugler, 1998).

1.1. Background

1.1.1. International status:

Numerous works have been conducted throughout the world in the isolation and identification of the bacteria belonging to the genera *Xenorhabdus* and *Photorhabdus* from their symbiotic nematodes including their pathogenecity.

The bacterial symbiosis isolated from the nematode *Steinernema carpocapse* were described for the first time in 1965 (Poinar and Thomas, 1965; Poinar and Thomas, 1966). They were named *Achromobacter nematophilus*, which was later transferred to a newly formed genus *Xenorhabdus* and renamed as *Xenorhabdus nematophilus*, which was then finally renamed as *Xenorhabdus nematophila* to conform with the bacterial classification (Thomas and Poinar, 1979; Euzéby and Boemare, 2000). Meanwhile, symbiotic glowing bacteria isolated from *Heterorhabditis bacteriophora* were included into the genus *Xenorhabdus* as *Xenorhabdus luminescens* (Thomas and Poinar, 1979). Hence, up to 1993, there were only two bacterial species in the genus *Xenorhabdus*, i.e. *Xenorhabdus nematophila* (type species) and *Xenorhabdus luminescens*, which comprised symbionts of *Steinernema* and *Heterorhabditis* nematodes, respectively (Akhurst, 1983; Akhurst and Boemare, 1988; Boemare and Akhurst, 1988). However, the significant differences in the phenotypic and molecular characters between these two species resulted in the reassign of all bacterial symbionts of *Heterorhabditis* into a new genus *Photorhabdus* as *Photorhabus luminescens* (Boemare et al., 1993). Since 2004, three new subspecies of *Photorhabdus* (Akhurst

et al., 2004) and 14 new species of *Xenorhabdus* (Lengyel et al., 2005; Tailliez et al., 2006) have been described. Recently, Machado et al, 2023 reclassified the symbiotic bacteria using the whole genome approach where *Photorhabdus* has 28 recognised taxa, including 22 species, six of which are further classified into subspecies. Machado et al.,(2023a) 28 taxa of *Xenorhabdus* associated with the nematodes have been identified so far including two recently identified subspecies Machado et al., (2023b).

Initially, some phenotypic features of the symbionts were the main criteria used to differentiate between *Xenorhabdus* and *Photorhabdus* bacteria and classify them into two separate groups (Thomas and Poinar, 1979). However, due to the limitations of morphology in characterizing the isolates, the 16s rRNA gene sequences-based phylogeny has proved to be useful for delineating species belonging to the genera *Photorhabdus* and *Xenorhabdus* (Liu et al., 1997; Tailliez et al., 2006, 2010) since the gene region is highly conserved in nature, universal, stable function, multiple copies and long gene sequence for informatics purposes (Woese et al., 1985; Janda and Abbott, 2007). The two groups of bacterial symbionts are clearly distinguished by the containment of unique sequences by *Photorhabdus* in the 16S small subunit rDNA, which *Xenorhabdus* lacks. The sequence TTCG of *Xenorhabdus* is at the 208–211 position (in terms of *E. coli* numbering), while *Photorhabdus* contains the longer TGAAG sequence (Szallas et al., 1997). However due to insufficiency of a single gene region used for determining phylogenetic relationship of the isolated bacteria, the RecA gene (encoding a DNA recombination protein) and the GyrB gene (encoding a DNA gyrase beta subunit) have been used by Tailliez et al., (2010). The amino acid sequences of GyrB are conservative enough to allow the comparison of taxa which are not closely related (Yamamoto and Harayama, 1996). The phylogenetic analyses of GyrB nucleotide sequences also reflected the evolutionary relationships of closely related species. The potential of phylogenetic studies using RecA of bacteria was first addressed by Lloyd and Sharp (1993). They have reported the reliability of RecA for shaping relationships among closely related bacterial species from the detailed analysis of the evolution of recA genes from 25 species of bacteria, they showed that phylogenetic trees of RecA proteins appeared to be reliable for determining relationships among closely related species of bacteria. These two gene were also used by several bacterial phylogeny by Eisen (1995); Akhurst et al. (2004); Thompson et al. (2004) and Wang et al. (2007). Various studies have shown that the *Photorhabdus* and *Xenorhabdus* bacteria are phylogenetically close and form a tight sister group in contrast to their EPN hosts *Steinernema* and *Heterorhabditis*, which represent distinct clades (Liu et al., 1997; Koppenhofer, 2007; Tailliez et al., 2010; Stock, 2015).

Various workers have been conducted numerous tests for determining the precise biochemical activity of the isolated bacteria. However, there has been considerable variations in biochemical reactions reported for *Xenorhabdus* and *Photorhabdus*, (Holt et al., 1994; Brenner and Farmer, 2005) This led to complications in defining the precise biochemical characteristics which may be due to several variable characters reported by different workers. The variation probably being a result of using different bacterial strains or phenotypic variants, choice of pH indicator, basal media used for the test (Boemare and Akhurst, 1988; Akhurst and Boemare, 1988; Holt et al., 1994). When the phenotypical characteristics of *Xenorhabdus* and *Photorhabdus* are considered, they can be seen to be out of character, compared to other members of the Enterobacteriaceae family (Holt et al., 1994). When comparing *Xenorhabdus* and *Photorhabdus*, two main differences are that the latter being catalase-positive, with the majority being bioluminescent while *Xenorhabdus* isolates are negative for both. (Poinar et al., 1980; Boemare and Akhurst, 1988).

The pathogenicity of bacterial symbionts has been tested against various economically important insect pests including insect vectors of diseases and pathogenic micro-organisms Benfarhat-Touzri

et al., 2014; Ahantarig, 2009; Vagelas et al., 2004). Apart from insecticidal activity, these bacteria were analysed for their antibacterial activity (Muangpat et al., 2017; 2020) antifungal activity on some phyto-pathogenic fungi (Chen et al., 1994; Orozco et al., 2018), nematicidal activity (Webster, 2002; Grundmann et al., 2014) and cytotoxicity against various cell lines (Dowling et al., 2004) with a promising outcome. All these properties have been attributed to the symbiotic bacteria, *Xenorhabdus* spp. and *Photorhabdus* spp, associated to the nematodes. Stock et al., (2017) performed a review on study of the secondary metabolites of *Photorhabdus* and compiled numerous bioactive secondary metabolites belonging to diverse chemical classes. Regardless of the large variation in bioactive compounds, none have been commercialized in chemical form.

1.1.2. National Status:

Work on entomopathogenic nematodes in India was started way back in 1966, but the works on its symbiotic bacteria gained interest recently. The information regarding the taxonomy of bacteria of the genera *Xenorhabdus* and *Photorhabdus*, their behavior, assay against insect pests and characterization at molecular level is still very limited in India. The native EPN, *Heterorhabditis indica* is associated with *Photorhabdus luminescens*, and another native *Steinernema*, *S. thermophilum* (junior synonym of *S. abbasi*) is found associated with a newly describe bacterium, *Xenorhabdus indica* (Somvanshi et al., 2006). Accordingly, local surveys, assay and genetic characterization of the bacteria were undertaken from ecologically diverse geographical regions of India. Recent investigations revealed the occurrence of *Steinernema sangi* (Lalramnghaki et al., 2017) and *Heterorhabditis baujardi* (Vanlalhlimpuia et al., 2018) in Mizoram which are associated with *Xenorhabdus vietnamensis* and *Photorhabdus luminescens akhurstii* respectively. In addition, a highly virulent strain of *P. luminescens akhurstii* was observed from Meghalya (Somvanshi et al.,2017).

To evaluate the native entomopathogenic bacteria against insect pests, Rajagopal and Bhatnagar (2002) applied *Photorhabdus luminescence* by direct injection to the insect larvae and observed the mortality of insects. Similarly, Mohan et al. (2003) evaluated the foliar application of *Photorhabdus luminescens* to kill the cabbage butterfly *Pieris brassicae* and reported the feasibility. The injection technique of the bacteria to insect was again performed by Inman et al. (2012) while characterizing the growth of *Photorhabdus luminescens* for the rearing of *Heterorhabditis bacteriophora*. Mahar et al. (2004) reported lethality of the cell and cell free filtrates of the *Xenorhabdus nematophila* isolated from *S. carpocapsae* against diamondback moth on Chinese cabbage leaves. Since bacteria of the genera *Xenorhabdus* and *Photorhabdus* are carried by nematodes to infect insect larvae, Rajagopal et al. (2006) focused on investigating the ability of *P. luminescens* in the soil environment to independently infect and kill insect larvae. They encapsulated *P. luminescens akhurstii* in sodium alginate beads and release in soil to assess their ability to kill *Spodoptera litura* 6th instar larvae upon feeding and proved the success of the experiment, paving the way for future formulations of the bacteria. Since then, work on the secondary metabolites of entomopathogenic bacteria was continuing with a good success rate. Vyas *et al*. (2008) evaluated the significance of native *Xenorhabdus* spp metabolites against collar rot and root knot disease of groundnuts and found a significant result. Bacterial broth of different strains of *Xenorhabus* [(*X.* assam-isolate (Sg as1), *X. indica* and *X.* Gujarat-isolate (SG gj)] was extracted with ethyl acetate where the potential activity of *X*. assam-isolate (Sg as1) exhibited highest antifungal activity against *M*. *phaseolina*. Aiswarya et al. (2017) reported the efficacy of ethyl acetate crude extracts of *Xenorhabdus stockiae* and *Photorhabdus luminescens* against selected pathogenic bacteria, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Klebsiella pneumonia* and suggest that they could be used as antibiotics in the future. Therefore,

entomopathogenic bacteria belonging to the genera *Xenorhabdus* and *Photorhabdus* may represent possible sources of novel compounds with effective antibacterial, antifungal and insecticides

It is thus evident that *Xenorhabdus* and *Photorhabdus* bacteria are an excellent source for novel antimicrobial metabolites, however, became a neglected antibiotic source (Pidot et al., 2014). Several workers revealed the significant potential of these bioactive secondary metabolites not only in vitro, but also in vivo in agricultural industry (Böszörményi et al., 2009; Fang et al., 2011). Therefore, identification and characterization of nematodes bacterial symbionts are crucial importance in an EPN-based biocontrol perspective (Emelianoff et al., 2008).

1.2. Study area:

Mizoram has an area of 21,087 sq. km and situated at 21° 58" N - 24° 35' N Latitude and 92° 15' - 93° 29' E Longitude. Mizoram is one of the biodiversity hotspots in the eastern Himalayan region (north east India) with about 94% tribal people living in the state**.** Entomopathogenic nematodes-bacteria were collected from 14 sites within the study area. (Fig. 1)

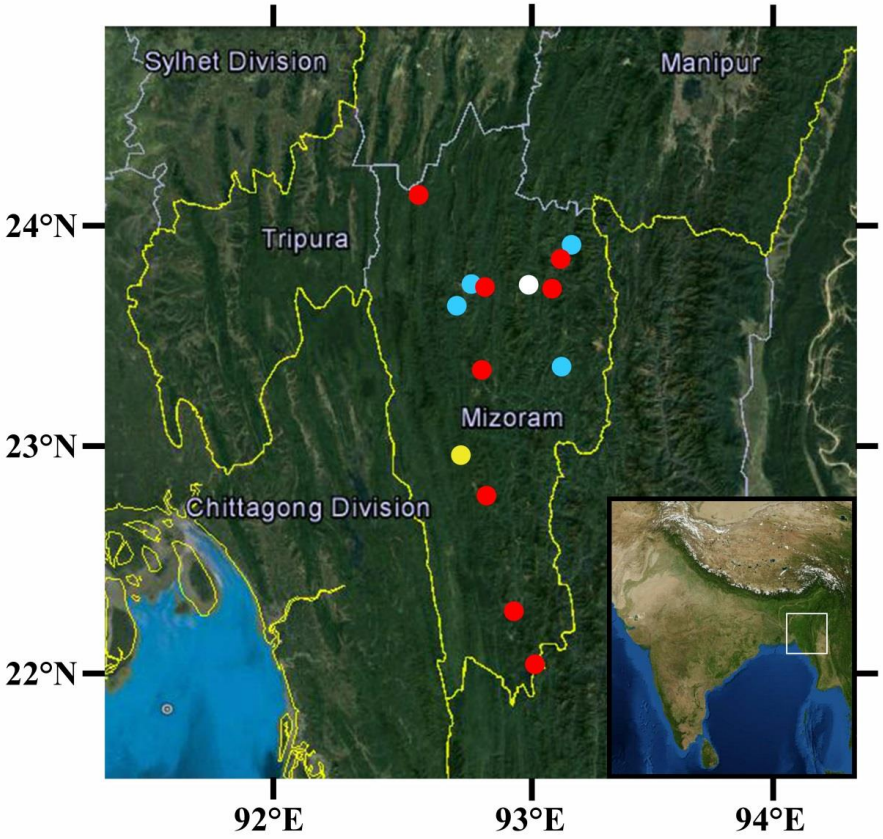

Figure 1: Study area-Map showing distribution of entomopathogenic nematodes/bacteria in Mizoram, NE India. *(Each circle represents more than one spot)*

- *Heterorhabditis baujardi/ P. hindustanensis*
- *Heterorhabditis indica/ P. namnaonensis*
- *Steinernema sangil X. vietnamensis*
- *Steinernema surkhetense/ X. surkhetense*

2 METHODOLOGIES, STARTEGY AND APPROACH

2.1. Nematode source:

Soil samples were collected randomly from different localities covering the 8 districts of Mizoram. The samples were collected from different habitats at a depth of 10 – 15 cms at each site covering an area about 1 sq. m. Nematodes were further isolated by baiting techniques (Bedding and Akhurst, 1975) where the last instar larvae of wax moth *Galleria mellonella* (L.) were used as baiting agent. Larval mortality was observed daily upto 10 days. The dead larvae were rinsed in distilled water and examined for the presence of entomopathogenic nematodes by colour change and smell emanating from the dead larvae. The infected larvae were transferred to modified White traps (Woodring and Kaya, 1988) and incubated at 25 °C. The emerged infected juveniles (IJs) were then collected for bacterial isolation.

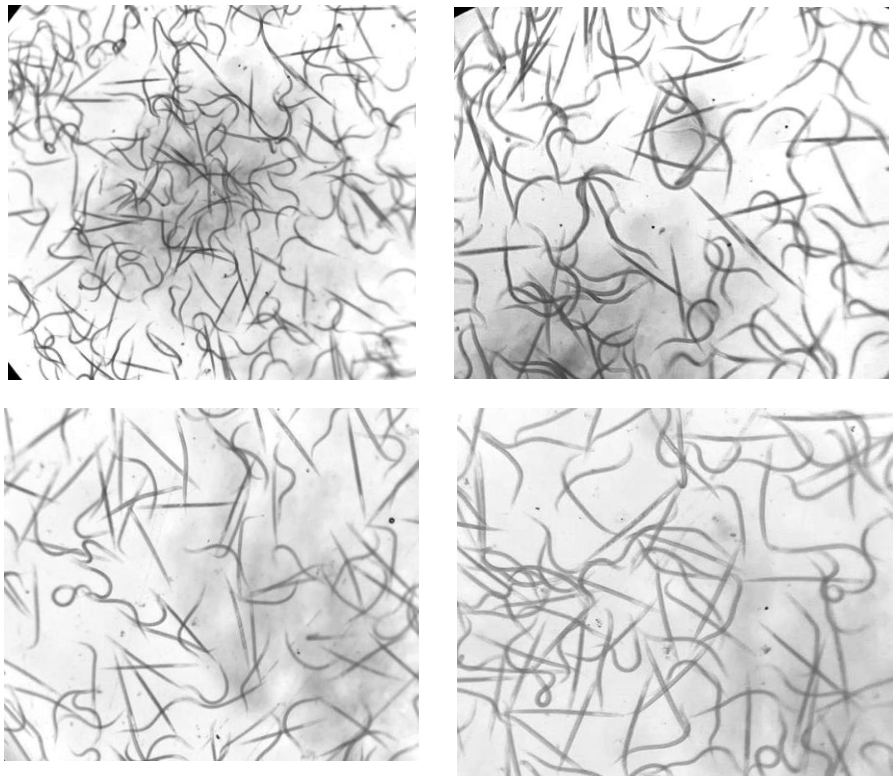

Figure 2. Nematode source

(A)-*Heterorhabditis indica*

(B)-*H. baujardi*

(C)- *Steinernema sangi*

(D)-*S. surkhetense*

2.2. Isolation and culture of bacterial symbionts from nematodes:

The primary forms of the bacteria were then isolated from freshly emerged infective juveniles of entomopathogenic nematodes by maceration in PBS buffer. Primary forms of the bacteria were isolated from freshly emerged infective juveniles of EPNs by maceration (Akhurst, 1980). The IJ's were collected in 1.5ml Eppendorf tube and washed with sterile Ringer solution with 10% sodium hypochloride (w/v) to avoid external contamination from the tegument. The collected lJ's were crushed in 1ml sterile PBS buffer lacking Mg^{2+} and Ca^{2+} salts (8 g NaCl, 0.2 g KCl, 1.15 g Na_2HPO_4, 0.2 g KH_2PO_4, 1 l H_2O sterile) and 100 microlitres was spread on NBTA medium

(Nutrient agar supplemented with 0.0025% bromothymol blue (w/v) and 0.004% Triphenyltetrazolium chloride (w/v) (Akhurst, 1980; Emelianoff et al, 2008). Growth is observed after 24–48 hours of incubation at 28℃. Single colony of bacteria which absorbs bromothymol blue dye was selected and streaked on nutrient agar media for storage, biochemical and morphological characterization. The code given to the isolates and their respective species are given in Table-1.

2.3. Phenotypic and biochemical characterization:

The absorbtion of dye on NBTA media was used as preliminary identification of the bacterial isolates (*Xenorhabdus* produces blue colour colonies on NBTA while *Photorhabdus* produces olive-green colour colonies). Bacterial isolates were sub-cultured on nutrient agar, incubated at temperature of 28 °C. After 24 hr of incubation, gram staining was performed using standard protocol. Motility of isolates were examined by observing the 24hr nutrient broth culture of bacteria under the microscope. Catalase activity was performed as per Reiner (2010). Citrate utilization, phenylalanine deaminase, indole production, methyl red and oxidase activities were analysed by following the methods of Cowan (2004). Production of acids from mannitol, lactose, maltose, raffinose, L-Arabinose and Rhamnose were conducted using phenol red as pH indicator and nutrient broth as basal medium. All the tests were conducted under aseptic conditions.

2.4. Molecular characterization and sequence analysis:

The genomic DNA was extracted using Phenol chloroform method and the 16S rRNA gene was amplified using primers 16SP1 (5′-GAAGAGTTTGATCATGGCTC-3′) forward and 16SP2 (5′-AAGGAGGTGATCCAGCCGCA-3′) reverse. Amplification using recA gene was done using primers RecA1F (5’-GCTATTGATGAAAATAAACA-3’) forward and RecA2R(5’-RATTTTRTCWCCRTTRTAGCT-3’) reverse direction. In addition, gyrase B gene (gyrB) was amplified using primers 1200FgyrB (5'- GATAACTCTTATAAAGTTTCCG-3') forward and 1200RgyrB (5'- CGGGTTGTATTCGTCACGGCC-3') reverse. The conditions applied for gene amplification were: 5 mins at 94 °C for denaturation followed by 35 cycles for 30 secs at 94 °C, 30 secs at 60 °C for annealing and 1 min at 72 °C for extension followed by 7 mins at 72 °C. The PCR conditions applied for recA were 5 min at 94 ℃ for denaturation followed by 30 cycles for 1 min at 94 ℃, 1 min at 55 ℃ for annealing and 1 min at 72 ℃ for extension followed by 5 min at 72 ℃. For gyrB, the PCR conditions were set as follows: 5 min at 94 ℃ for denaturation followed by 30 cycles for 1 min at 94 ℃, 45 s at 58 ℃ for annealing and 2 min at 72 ℃ for extension followed by 7 min at 72 ℃. The PCR products were sequenced in forward direction at Scigenom Kochi, Kerala. The generated sequences were edited and aligned, which were further blasted in NCBI for the nearest matches and the developed sequences will be submitted to NCBI GenBank. The resulting sequences were edited using FinchTV 1.4.0 software packages (Geospiza, Inc.; Seattle, WA, USA; http://www.geospiza.com) and aligned using Clustal X 1.64 (Thompson et al., 1997). The phylogenetic relationships were established using Maximum Likelihood (ML), in MEGA X (Kumar et al., 2018) with the selected species sequences retrieved from GenBank. Bootstrap analysis was carried out with 1000 datasets.

2.5. Insecticidal activity:

Insecticidal activity was done by following the method of Vitta et al. (2018) with modification. *Galleria mellonella* was selected as the model organism to screened insecticidal activity of the bacterial isolates and the bacterial isolates were used to study the activity of symbionts towards larvae of *Galleria mellonella.* The larvae of *Galleria mellonella* were collected from local bee keeper. The larvae were maintained in their natural diets (honeycombs). Male and Female moths were kept in the oviposition cage for egg laying. The cages were kept at 30°C in a dark place. The eggs were transferred to the artificial diet and reared by modification of Singh (1997). For

insecticidal activity, 48 hours culture cell suspensions was diluted using distilled water and adjusted to 1.0 OD at 600nm using spectrophotometer. A unit of 1ml of the adjusted concentrations wes dropped on a small petridish containing the larvae of *Galleria mellonella.* Larval mortality rate was observed during 24 to 96 hours of exposure. The experiment was performed in triplicate and distilled water was used as negative control.

2.6. Antibacterial activity:

2.6.1. Preparation of pathogenic bacteria

Four strains of pathogenic bacteria, viz. *E. coli* (ATCC 10536), *K. pneumoniae* (ATCC 10031), *P. aeruginosa* (ATCC 10145), and *B. subtilis* (ATCC 11774) were collected from the Regional Institute of Paramedical and Nursing Sciences (RIPANS), Aizawl, Mizoram, North-east India were used for the experiment. A single colony was transferred to the nutrient broth and incubated for 24 h at 30 ℃. The concentration of the overnight grown culture was adjusted to 0.08 – 1.00 O. D (0.5 McFarland standard) using a spectrophotometer at a wavelength of 600 nm.

2.6.2. Preparation of bacterial extract

The extraction of bacterial metabolites and preparation of stock concentrations were prepared according to Muangpat et al. (19) with a slight modification. The whole-cell suspension of the bacterial isolates was used for preliminary screening of antibacterial activity. 50 µl of the cell suspension was incorporated into the agar well containing the spread pathogenic strain and incubated at 30°C for 24 h. A clear zone surrounding the well was read as an inhibition zone. For all the bacterial isolates, a single colony was transferred to a 1000 ml sterile nutrient broth and stored at 28 ℃ in a shaker incubator for 48 h which was subsequently transferred to 2000 ml of separating funnel. To extract the crude compound, the same volume of ethyl acetate was added and mixed well by inverting the funnel. The mixture was kept at room temperature for 24 h. The ethyl acetate layer was collected and further evaporated using a rotary vacuum evaporator (Rotavapor® R-100 System-Buchi, Switzerland). The extraction procedure was performed thrice to maximize the amount of crude extract.

A total of 500 mg of the condensed bacterial extract was dissolved in 1 ml of DMSO and kept as a stock solution. A unit of 10 µl from the stock solution was pipetted out and impregnated into 6 mm paper disks. The paper disks were then placed on Mueller Hinton Agar (MHA) previously plated with the selected pathogenic strain. The plates were incubated at 30 ℃ for 24 h. The diameter of a clear inhibition zone was measured in millimeters using a ruler. A drop of DMSO was used as negative control and an ampicillin disk was used as a positive control.

2.6.3. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC of the bacterial extracts was performed using the microdilution method in a 96-well microtiter plate. The same volume of bacterial extracts and sterile Mueller Hinton Broth (MHB) was mixed in a well followed by two-fold serial dilutions. Then, each well was inoculated with 10 µl of microbial inoculum initially adjusted to 0.5 Mc Farland standard. After mixing well, the plates were incubated at 30 °C for 24 h. A mixture of DMSO and nutrient broth with inoculum and the same mixture without inoculum were used as control. The MIC was determined as the lowest concentration from each extract with a clear well as detected by the unaided eye.

For MBC, 10 µl of the extract dilution representing the MIC including two more concentrated diluted wells were streaked on MHA to observe visible growth. The plates were incubated at 30 °C for 24 h and growth was observed corresponding to different concentrations. The MBC was determined as the lowest concentration of bacterial extracts with no visible growth on MHA.

2.6.4. Statistical data analysis

The diameter of the inhibition zone was presented in the form of Mean ± Standard Error of Mean (SEM). One-way analysis of variance (ANOVA) was performed to determine a variation of inhibition exhibited by the bacterial isolates (at the level of $P \leq 0.05$) against the selected pathogenic bacteria.

2.7. Antifungal activity:

The test organisms (*F. oxysporum*, *F. solani* and *C. albicans*) were collected from Research Centre, Pachhunga University College. A 10 µl of 48 hr old bacterial culture adjusted to 3×10^7 CFU ml^{-1} was spread using a sterile spreader over 9 cm petri-plate containing potato dextrose agar. About 4 mm mycelium of the test fungus were cut from the culture grown on PDA and were subsequently placed at the center of PDA plate containing bacterial symbiont. Control plate consisted of PDA lacking bacterial symbiont. The diameter of fungal growth in each plate were observed and compared with control plate every 48 hr for a period of one week depending on the growth of test fungus. The percent inhibition of the fungus was calculated by the following formula (Balouiri et al., 2016):

Antifungal activity (%) = ((Dc – Ds)/Dc) × 100

where, Dc is the diameter of fungal growth in control plate and Ds is the diameter of fungal growth in the plate containing tested bacterial isolates.

3. KEY FINDINGS AND RESULTS

3.1 Phenotypic and biochemical characteristics of the isolates:

Two species of *Xenorhabdus* bacteria, viz. *X*. *vietnamensis* from *Sternernema sangi* and *X*. *stockiae* from *S*. *surkhetense* and two species of *Photorhabdus*, viz. *P*. *hindustanensis* and *P. namnaonensis* from *H*. *indica* and *H*. *baujardi* respectively were identified. Steinernematids are associated with *Xenorhabdus* spp. and heterorhabditids with *Photorhabdus* spp. All bacterial isolates grew equally well on dark, NBTA media at 28 °C. *Xenorhabdus* isolates on absorbing bromothymol blue produced blue coloured colonies, while *Photorhabdus* isolates produced blue green coloured colonies. The absorption of dye on NBTA was used as preliminary identification of the bacterial isolates. Moreover, a single colony from NBTA plate was further sub cultured on nutrient agar media which showed distinct colouration between the genera. (Figure 2) Both the *Photorhabdus* isolates (TD and TS) showed yellow pigment whereas both the *Xenorhabdus* isolates (RF and PTS) showed off-white colour on nutrient agar. Biochemical characterization of the bacterial isolates was shown in Table 2.

Table 1: Bacterial symbionts and their associated entomopathogenic nematodes included in the study.

Bacterial isolates	Nematode host	Bacterial species	Source
RF	*Steinernema sangi*	*Xenorhabdus vietnamensis*	Mizoram, India
PTS	*Steinernema surkhetense*	*Xenorhabdus stockiae*	Mizoram, India
TD	*Heterorhabditis baujardi*	*Photorhabdus namnaonensis*	Mizoram, India
TS	*Heterorhabditis indica*	*Photorhabdus hindustanensis*	Mizoram, India

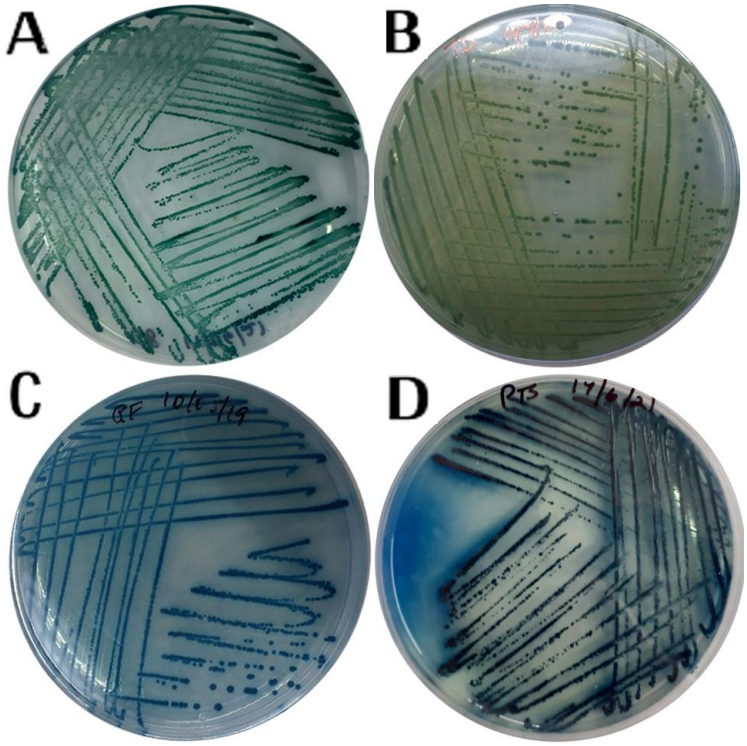

Figure 3: Pure culture of symbiotic bacteria on NBTA media**:**

(A)-*Photorhabdus hindustanensis*(TS) isolated from *Heterorhabditis indica*

(B)-*Photorhabdus namnaonensis* (TD) isolated from *Heterorhabditis baujardi*

(C)- *Xenorhabdus vietnamensis* (RF) isolated from *Steirnernema sangi*

(D)- *Xenorhabdus stockiae* isolated from *Steirnernema surkhetense*

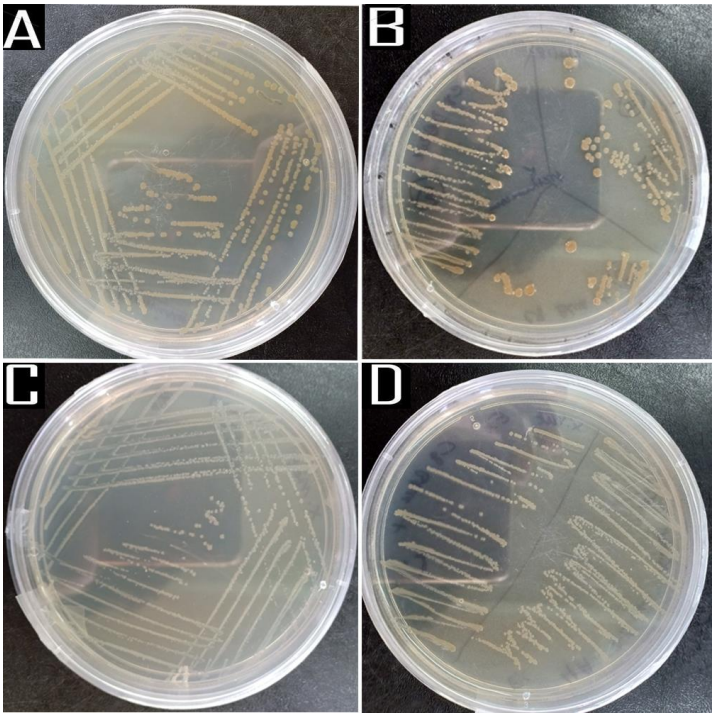

Figure 4: Pure culture of symbiotic bacteria on Nutrient Agar (NA) media**:**

(A)-*Photorhabdus hindustanensis* (TS) isolated from *Heterorhabditis indica*

(B)-*Photorhabdus namnaonensis* (TD) isolated from *Heterorhabditis baujardi*

(C)- *Xenorhabdus vietnamensis* (RF) isolated from *Steirnernema sangi*

(D)- *Xenorhabdus stockiae* (PTS) isolated from *Steirnernema surkhetense*

Table.2: Biochemical characterization of the bacterial isolates.

Name of the test	*Xenorhabdus* sp.		*Photorhabdus* sp.	
	RF	PTS	TD	TS
Gram stain	–	–	–	–
Shape	Rod	Rod	Rod	Rod
Motility	Motile	Motile	Motile	Motile
Catalase	–	–	+	+
m-inositol	–	–	–	–
Citrate utilization	–	–	+	+
Phenylanaline deaminase	–	–	–	–
Indole production	–	–	–	–
Methyl Red	–	–	+	+
Oxidase	–	–	–	–
Mannitol	+	+	+	+
Lactose	–	–	–	–
Maltose	+	+	+	+
Raffinose	–	–	–	–
L-Arabinose	–	–	–	–

Rhamnose	–	–	–	–
Lysine decarboxylase	–	–	–	–
Pigmentation	Off-white	Off-white	Yellow	Yellow

3.2. Molecular characterization:

The total length of the 16S rRNA sequence developed is 1192 bp. The two *Photorhabdus* isolates, TS and TD exhibited 98.73% similarity (1.4% K2P distance) between them. On comparison with other closely related type strain from NCBI database, TS isolates exhibited 99. 60% similarity (0.3% K2P distance) with the database sequence of P. *hindustanensis* (JX221722) while TD isolates exhibited 99.24% similarity (0.7% K2P distance) with *P. namnaonensis* (MK039087). Further comparison indicates that TS isolates showed 99.46% similarity (0.4% K2P distance), 99.24% similarity (0.42% K2P distance), 98.99% similarity (0.9% K2P distance) with *Photorhabdus aegyptia* (JAGJDU01) with *P. akhurstii* subsp. *akhurstii* (MK039086) and *P. hainanensis* (MK039085) respectively. TD isolates exhibited 98.49% similarity (1.3% K2P distance), 98. 50 % similarity (1.7% K2P distance), 98.32% similarity (1.7% K2P distance) with *P. akhurstii* subsp. *akhurstii* (MK039086), *P. hindustanensis* (JX221722), *P. luminescens* subsp. *venezuelensis* (OP727818) respectively.

The two *Xenorhabdus* isolates RF and PTS exhibited 96.49% (4.5% K2P distance) similarity between them. The BLAST search result of NCBI GenBank showed that the isolate, RF isolate have 99.8% similarity with the database sequence of *X. vietnamensis* (NR115713) with 0.01% K2P distance while PTS isolates exhibit 99.35% similarity (0.8% K2P distance) with *X. stockiae* (DQ202309). On comparison with other closely related species from NCBI GenBank, RF isolate showed 98.59% similarity (1.4% K2P distance) and 96.94% similarity (3.2% K2P distance) with *X. japonica* (EU934513) and *X. doucetiae* (DQ211709) respectively while PTS showed 97.41% similarity (3.0% K2P distance) 97.25% similarity (3.2% K2P distance) with *X. innexi* (AJ810292) and *X. kozodoii* (EU934522).

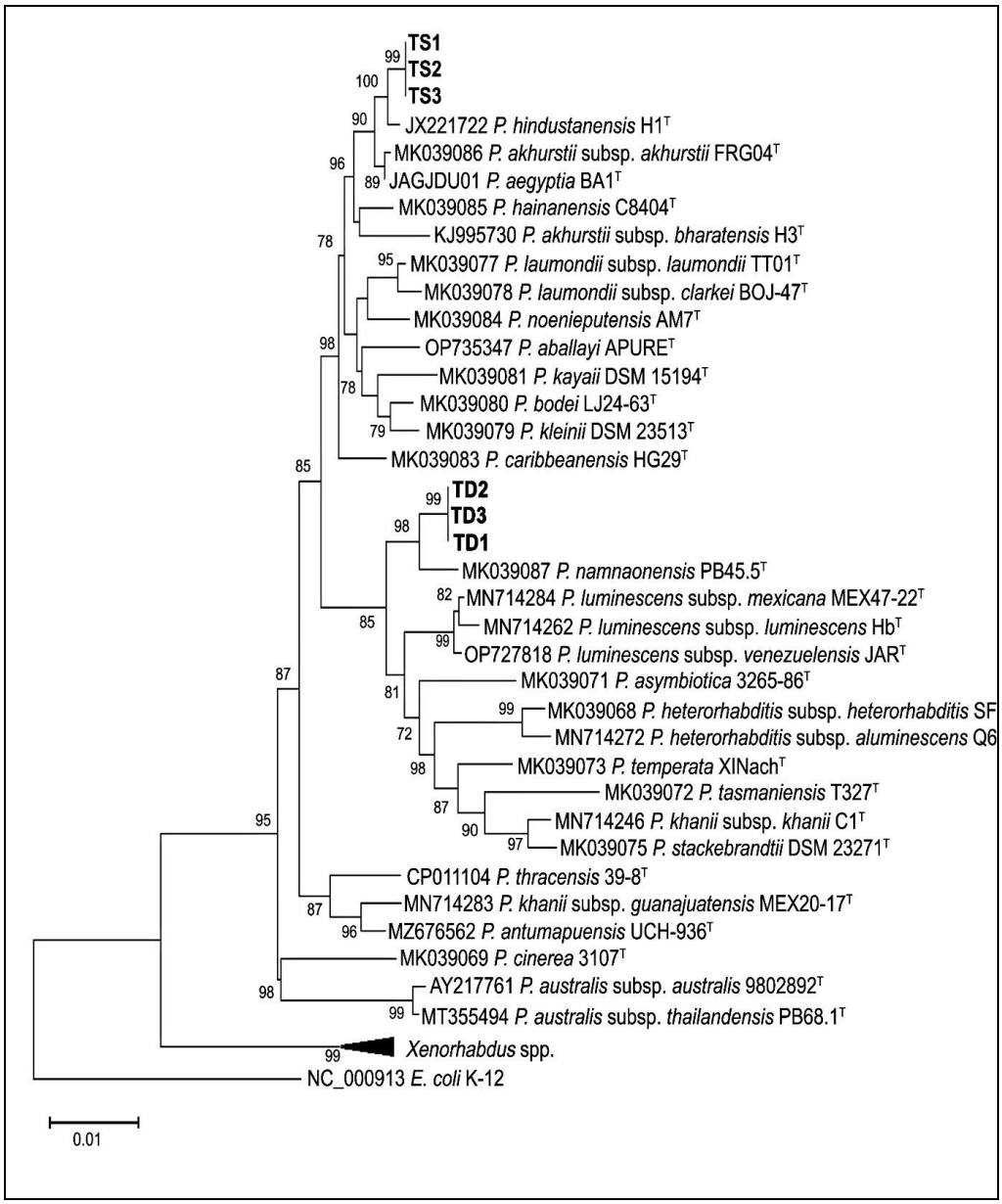

Figure 5: Maximum likelihood tree of *Photorhabdus* isolates inferred from **16S rRNA** gene. The numbers at the nodes correspond to the bootstrap support (1000 replicates, 50% or more). GenBank accession numbers and strain codes were given along with each species.

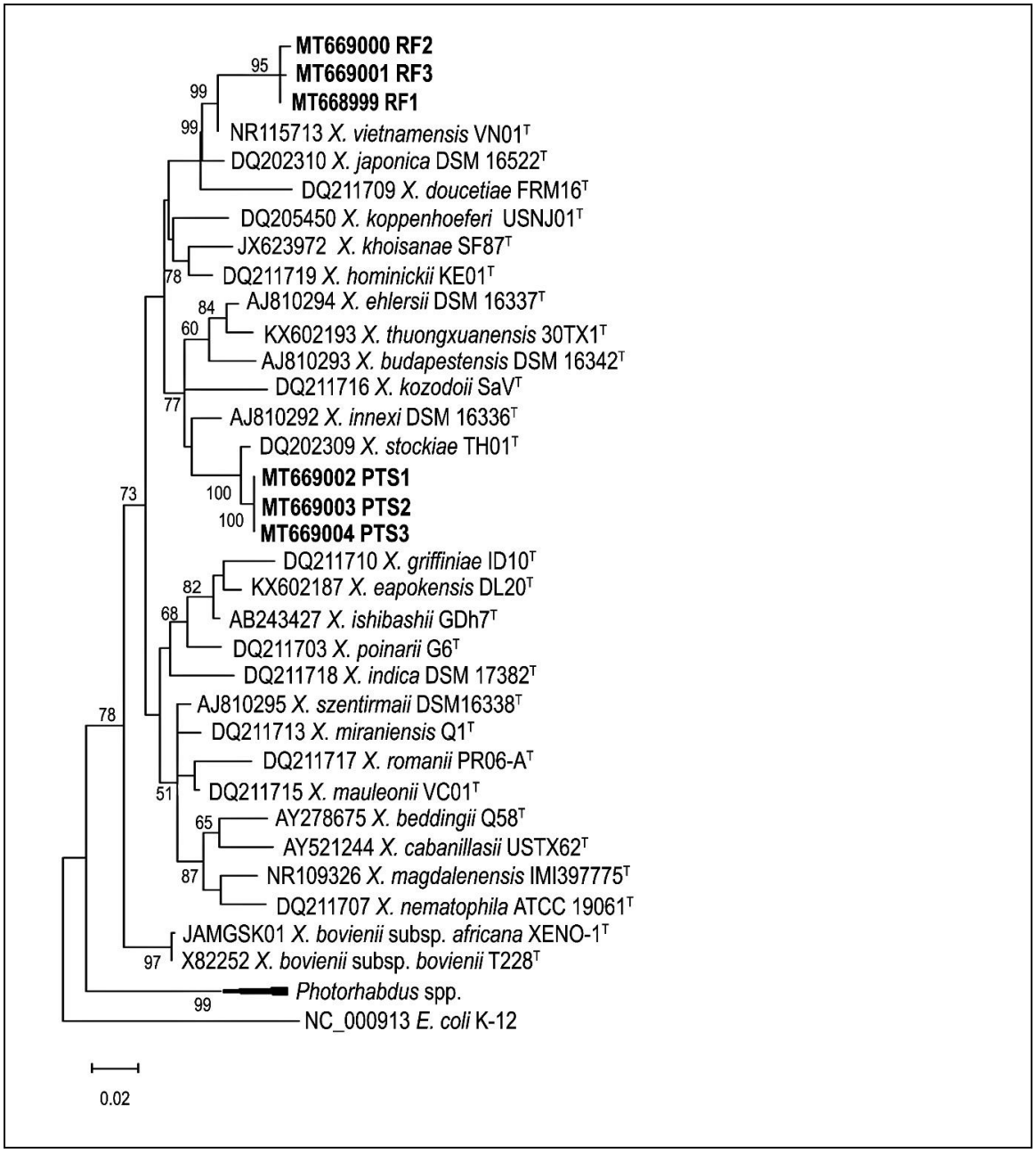

Figure 6: Maximum likelihood tree of *Xenorhabdus* isolates inferred from **16S rRNA** gene. The numbers at the nodes correspond to the bootstrap support (1000 replicates, 50% or more). GenBank accession numbers and strain codes were given along with each species.

The total length of the recA sequence developed is 860 bp. The two isolates, TS and TD, consistently exhibited 98.1% similarity (2.3% K2P distance) between them. Further, based on the analysis of the developed gene region with closely related type species from the GenBank, the *Photorhabdus* isolates (TS) exhibited a closer relationship with *P. hindustanensis* (PUWT01) (99.53% similarity with 0.8% K2P distance). Another *Photorhabdus* isolate (TD) showed 98.59% (1.6% K2P distance) similarity with *P. namnaonensis* (LOIC01). Further analysis showed that the *Photorhabdus* (TS) isolate exhibited 99.41% (0.9% K2P distance), 98.24% (2.1% K2P distance) and 98.13% (2.2% K2P distance) similarity with *P. akhurstii* subsp. *akhurstii* (RCWE01), *P. hainanensis* (RCWD01) and *P. akhurstii* subsp. *bharatensis* (RCWU01) respectively, whereas, the *Photorhabdus* isolate (TD) exhibited 98% (2.3% K2P distance), 97.83% (2.4% K2P distance), and 96.9% (3.2% K2P distance) similarity with *P. hindustanensis* (PUWT01), *P. akhurstii* subsp. *akhurstii* (RCWE01) and *P. hainanensis* (RCWD01) respectively.

Simultaneously, the two *Xenorhabdus* isolates, RF and PTS exhibited highest similarity with *X. vietnamensis* (FJ823401) (99.69% similarity with 0.31% K2P distance) and *X. stockiae* (KX826948) (99.41% similarity with 0.6% K2P distance) respectively. There is no significant similarity between RF and PTS isolates. Further, the isolates, RF showed 95.50% similarity (5.0% K2P distance) 94.10% (6.6% K2P distance) with *X. japonica* (FJ823400), 94.11% (6.6% K2P distance) similarity with *X. griffiniae* (FJ823399) and 94.11% similarity (6.6% K2P distance) with *X. ehlersii* (FJ823398) while PTS isolates showed 96.10% similarity (4.4% K2P distance) with another *X. stockiae* isolate (FJ823425) and 93.21% similarity (7.0% K2P distance) with *X. innexi* (FJ823423).

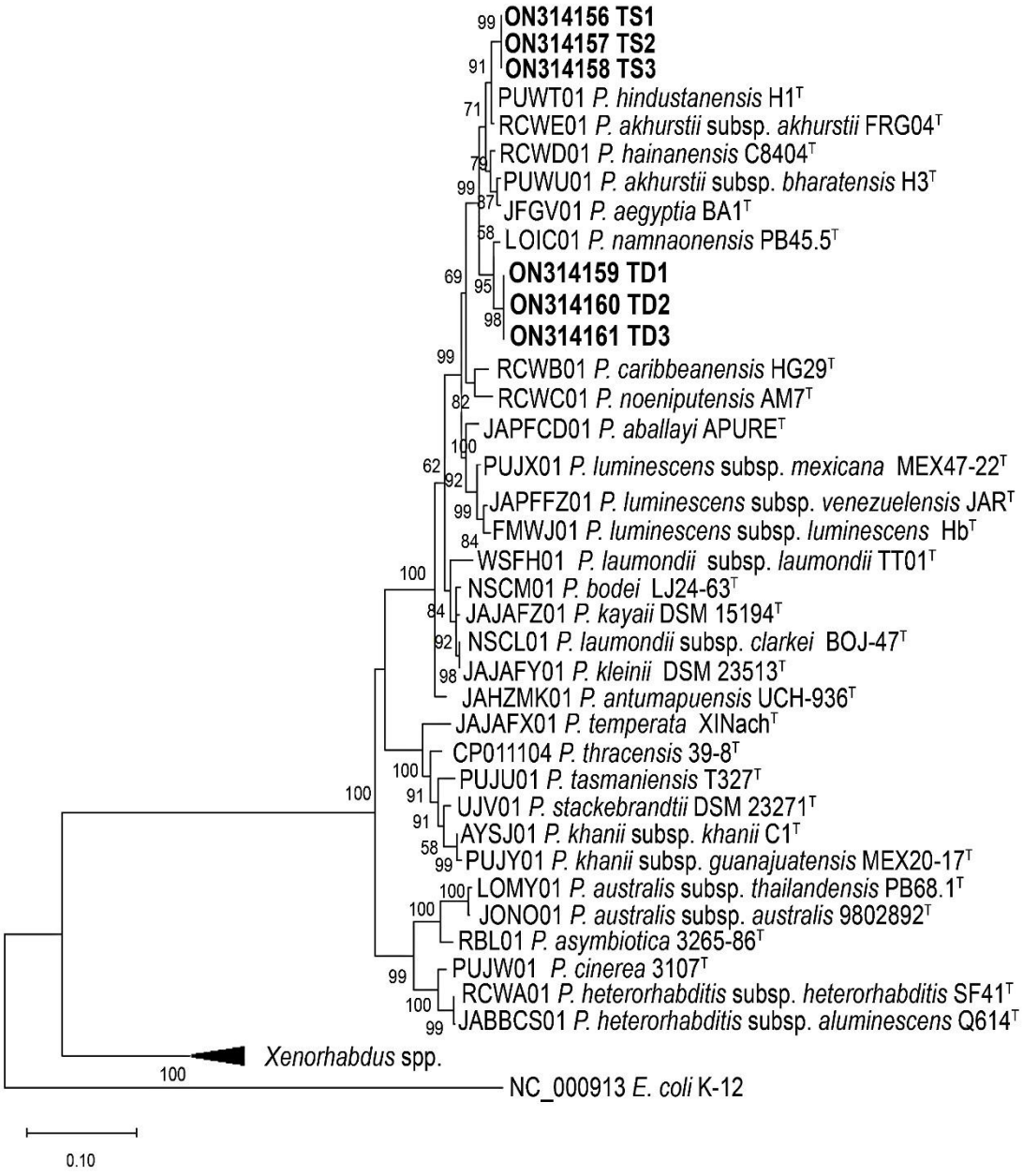

Figure 7: Maximum likelihood tree of *Photorhabdus* isolates inferred from **recA** gene. The numbers at the nodes correspond to the bootstrap support (1000 replicates, 50% or more). GenBank accession numbers and strain codes were given along with each species.

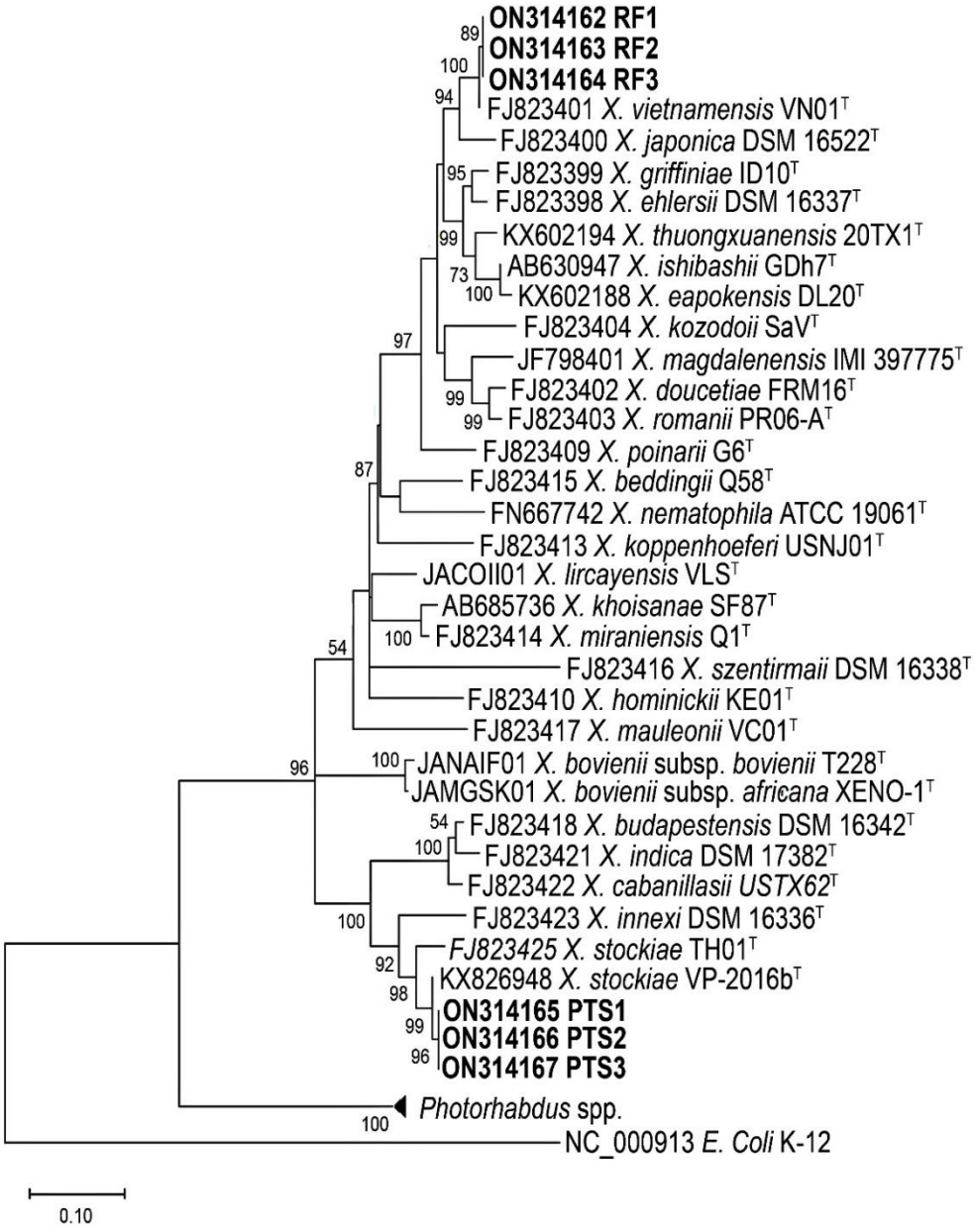

Figure 8: Maximum likelihood tree of *Xenorhabdus* isolates inferred from **recA** gene. The numbers at the nodes correspond to the bootstrap support (1000 replicates, 50% or more). GenBank accession numbers and strain codes were given along with each species.

The total length of the gyrB sequence developed is 1050 bp. The two *Photorhabdus* isolates, TS and TD showed 96.88% similarity (3.2% K2P distance) between them. Further analysis of the isolates and comparison with the type species on NCBI GenBank showed that the *Photorhabdus* isolates (TS) exhibited 99.43% (0.5% K2P distance) similarity with *P. hindustanensis* (PUWT01), whereas, *Photorhabdus* (TD) isolates exhibited the highest similarity with *P. hainanensis* (RCWD01) (98.30% similarity with 1.7% K2P distance. Comparisons with other closely related species shows that *Photorhabdus* isolates (TS) exhibited 97.83% (2.2% K2P distance), 96.60% (3.5% K2P distance), 97.17% (2.9% K2P distance) similarity with *P. akhurstii* subsp. *akhurstii, P. akhurstii* subsp. *bharatensis* (RCWU01) and *P. hainanensis* (RCWD01) respectively, whereas, the *Photorhabdus* (TD) isolates showed 97.26% (2.8% K2P distance), 96.22% (2.8% K2P distance) and 96.88%

(3.3% K2P distance) similarity with *P. namnaonensis* (LOIC01) and *P. akhurstii* subsp. *akhurstii* (RCWE01) and *P. hindustanensis* (PUWT01) respectively.

Simultaneously, the *Xenorhabdus* isolates, RF and PTS, showed 88.96% (17% K2P distance) between them. RF and PTS isolates exhibited 99.88% (0% K2P distance) and 99.15% (0.8% K2P distance) similarity with the database sequence of *X. vietnamensis* (EU934514) and *X. stockiae* (KX826949) respectively. Further, among the *Xenorhabdus* isolates, RF showed 95.48% similarity with *X. japonica* (EU934513) and PTS showed 97.45% (2.6 K2P distance), 93.97% similarity (7.0% K2P distance) similarity with *X. stockiae* (EU934542) and *X. innexi* (EU934540) respectively.

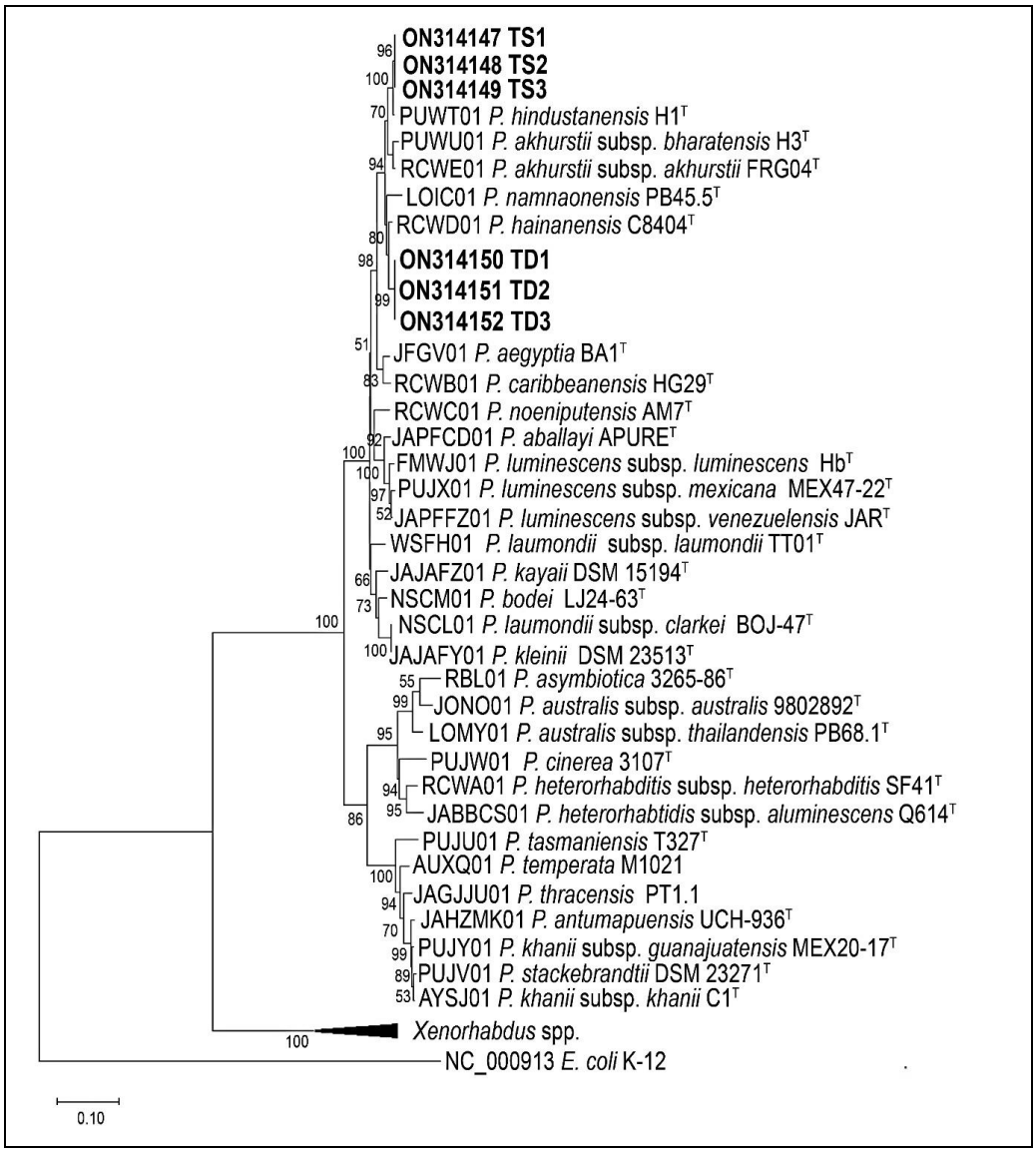

Figure 9: Maximum likelihood tree of *Photorhabdus* isolates inferred from **gyrB** gene. The numbers at the nodes correspond to the bootstrap support (1000 replicates, 50% or more). GenBank accession numbers and strain codes were given along with each species.

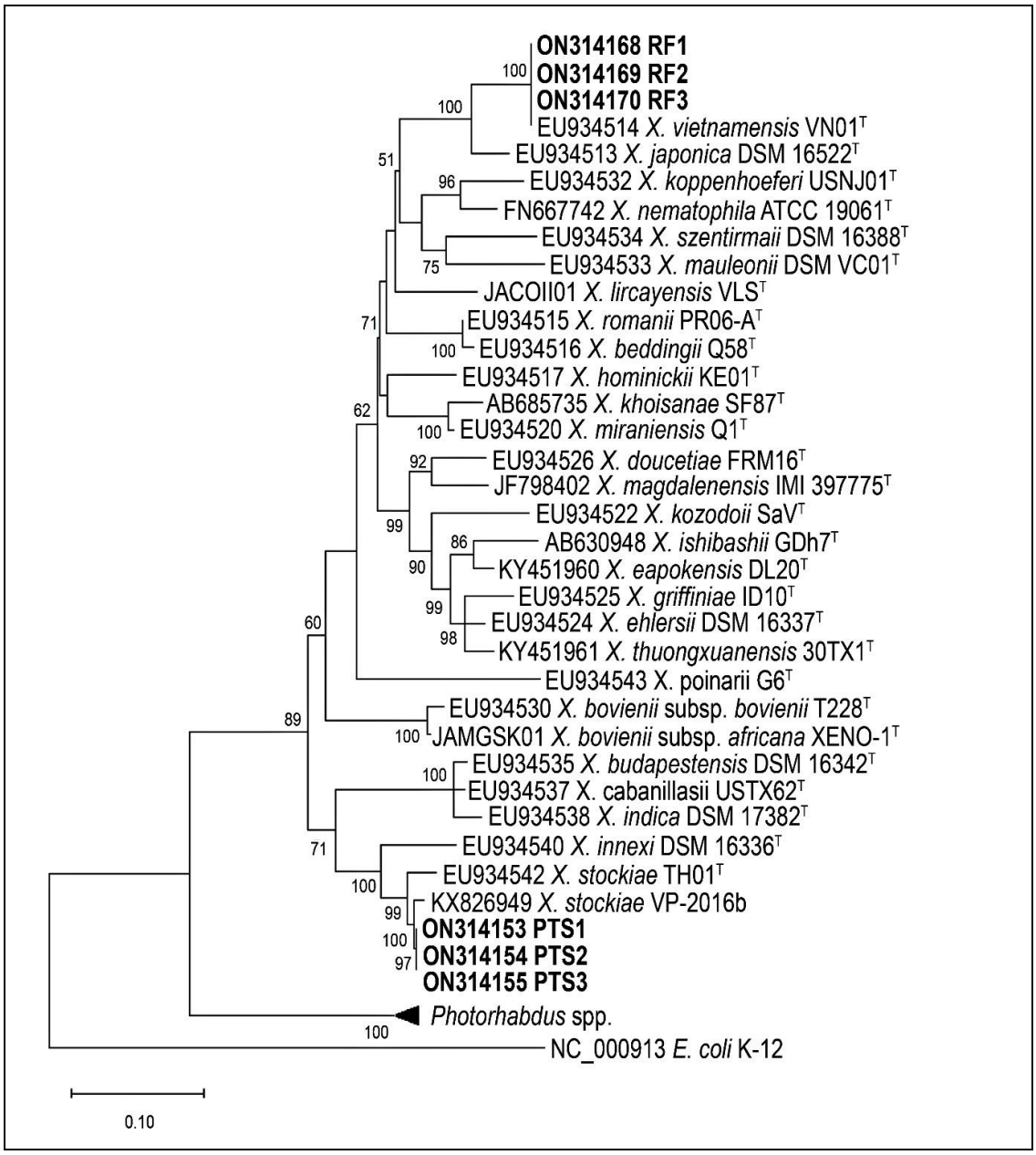

Figure 10: Maximum likelihood tree of *Xenorhabdus* isolates inferred from **gyrB** gene. The numbers at the nodes correspond to the bootstrap support (1000 replicates, 50% or more). GenBank accession numbers and strain codes were given along with each species.

The ML tree was constructed from the concatenation of three nucleotide sequences (16S rRNA, recA and gyrB genes) showed that the *Photorhabdus* isolates, TS and TD exhibited 2% K2P distance between them. Detailed analysis with closely related species showed that TS and TD isolates clustered cohesively with the type strain of *P. hindustanensis* H1^T (0.5% K2P distance) and *P. namnaonensis* PB45.5^T (1.7%) respectively. On further comparison, TS showed 1.1%, 1.2% and 1.8% K2P distance with *P. akhurstii* subsp. *akhurstii* (FRG04^T), *P. akhurstii* subsp. *bharatensis* (H3^T) and *P. aegyptia* (BA1^T).

The *Xenorhabdus* isolates, RF and PTS exhibited 11% K2P distance between them. On further analysis with closely related species, RF and PTS isolates are closely related with the type strain of *X. vietnamensis* VN01^T (0.1% K2P distance) and *X. stockiae* TH01^T (2%

K2P distance) respectively. On further comparison with other related species, RF isolates showed 9.0% K2P distance with *X. japonica* (DSM 16522) while PTS isolates showed 5.3% and 7.7% K2P distance with *X. innexi* (DSM 16336), *X. budapestensis* (DSM 16342) and 8.3% K2P distance with *X. indica* (DSM 17382) respectively. The overall mean distance of the concatenated sequences was 10 % with a standard error of 0.00.

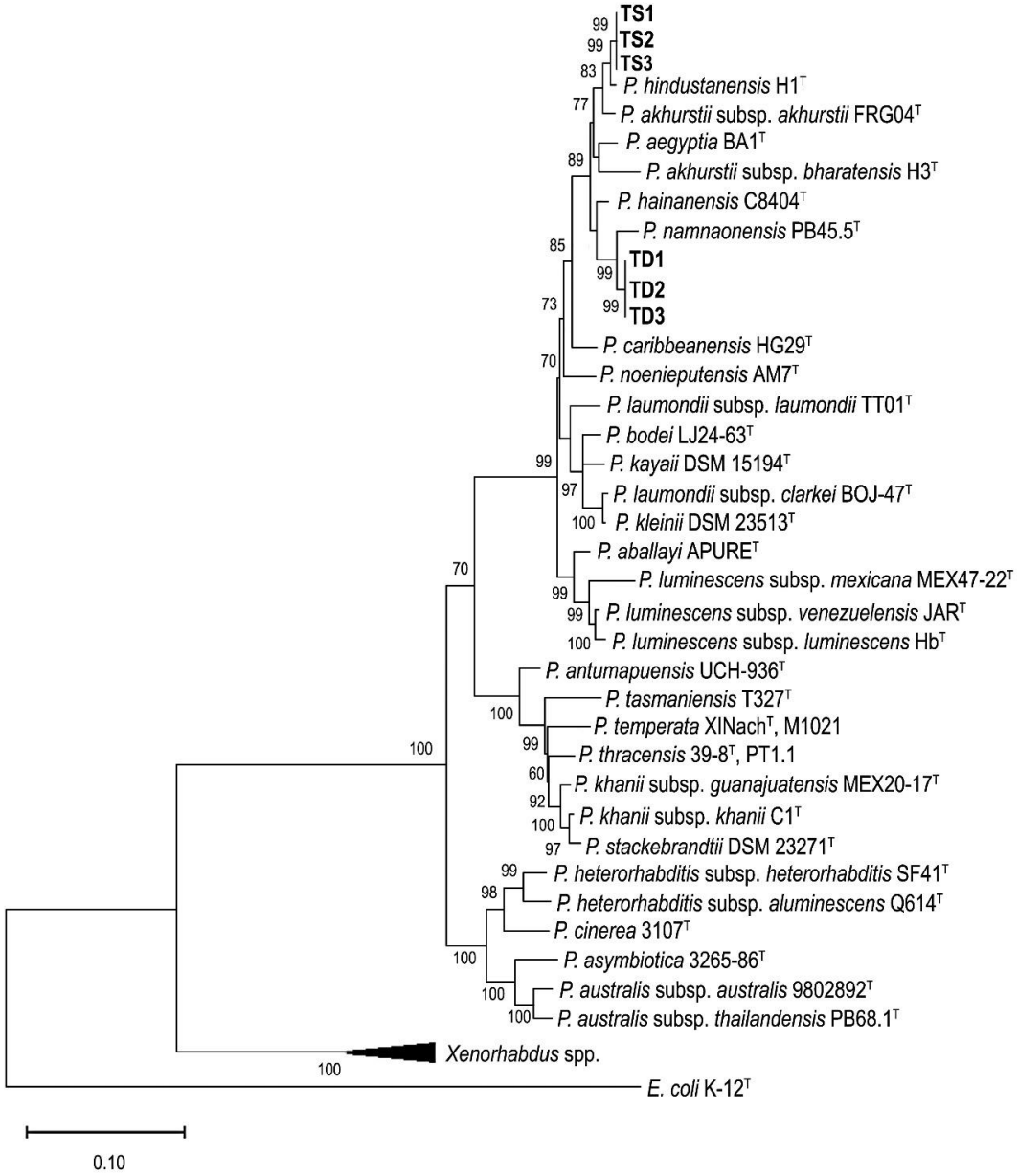

Figure 11: Maximum likelihood tree of *Photorhabdus* isolates inferred from concatenated gene (**16S, recA and gyrB)** gene. The numbers at the nodes correspond to the bootstrap support (1000 replicates, 50% or more). GenBank accession numbers and strain codes were given along with each species.

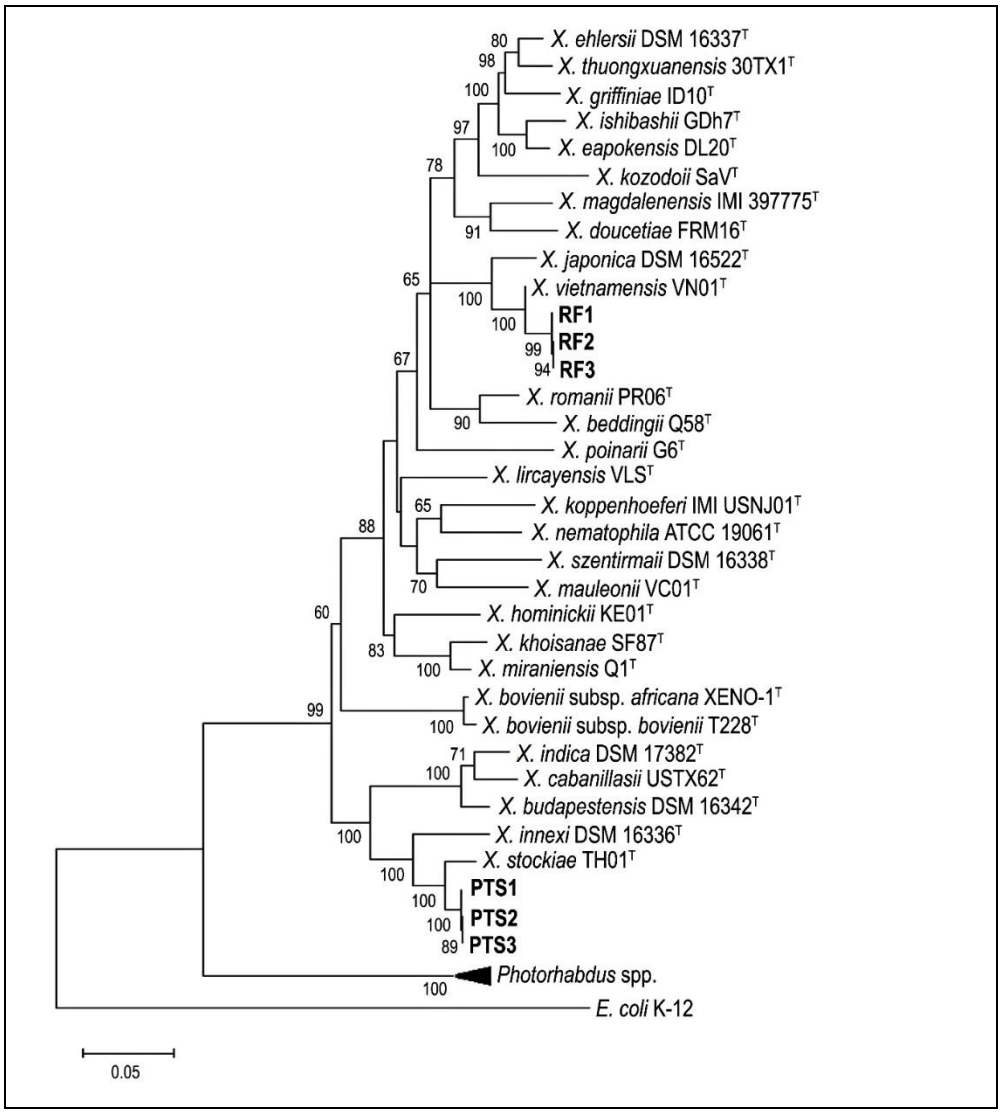

Figure 12: Maximum likelihood tree of *Xenorhabdus* isolates inferred from concatenated gene (**16S, recA and gyrB)** gene. The numbers at the nodes correspond to the bootstrap support (1000 replicates, 50% or more). GenBank accession numbers and strain codes were given along with each species.

Table 3. Sequences analysed in this study

Strain	**Species**	**Nematode Host**	**NCBI Accession No. (16S rRNA)**	**NCBI Accession No. (recA)**	**NCBI Accession No. (gyrB)**	**Country**	**References**
TS1	*Photorhabdus hindustanensis*	*H. indica*	**MT668996**	**ON314156**	**ON314147**	**Mizoram, India**	**This study**
TS2	*Photorhabdus hindustanensis*	*H. indica*	**MT668997**	**ON314157**	**ON314148**	**Mizoram, India**	**This study**
TS3	*Photorhabdus hindustanensis*	*H. indica*	**MT668998**	**ON314158**	**ON314149**	**Mizoram, India**	**This study**
TD1	*Photorhabdus namnaonensis*	*H. baujardi*	**MT668993**	**ON314159**	**ON314150**	**Mizoram, India**	**This study**
TD2	*Photorhabdus namnaonensis*	*H. baujardi*	**MT668994**	**ON314160**	**ON314151**	**Mizoram, India**	**This study**
TD3	*Photorhabdus namnaonensis*	*H. baujardi*	**MT668995**	**ON314161**	**ON314152**	**Mizoram, India**	**This study**
RF1	*Xenorhabdus vietnamensis*	*S. sangi*	**MT668999**	**ON314162**	**ON314168**	**Mizoram, India**	**This study**
RF2	*Xenorhabdus vietnamensis*	*S. sangi*	**MT669000**	**ON314163**	**ON314169**	**Mizoram, India**	**This study**
RF3	*Xenorhabdus vietnamensis*	*S. sangi*	**MT669001**	**ON314164**	**ON314170**	**Mizoram, India**	**This study**
PTS1	*Xenorhabdus stockiae*	*S. surkhetense*	**MT669002**	**ON314165**	**ON314153**	**Mizoram, India**	**This study**
PTS2	*Xenorhabdus stockiae*	*S. surkhetense*	**MT669003**	**ON314166**	**ON314154**	**Mizoram, India**	**This study**
PTS3	*Xenorhabdus stockiae*	*S. surkhetense*	**MT669004**	**ON314167**	**ON314155**	**Mizoram, India**	**This study**
MEX47-22^T	*Photorhabdus luminescens* subsp. *mexicana*	*H. mexicana*	MN714284	PUJX01	PUJX01	Mexico	Machado *et al.* (2019, 2021)
ATCC 9999^T	*P. luminescens* subsp. *luminescens*	*H. bacteriophora*	MN714262	FMWJ01	FMWJ01	Australia	Machado *et al.* (2019, 2021)
JAR^T	*P. luminescens* subsp. *venezuelensis*	*H. amazonensis*	OP727818	JAPFFZ01	JAPFFZ01	Venezuela	Machado *et al.* (2023)
DSM 3369 ^{T}	*P. khanii* subsp. *khanii*	*H. bacteriophora*	MN714246	AYSJ01	AYSJ01	USA	Machado *et al.* (2019)
MEX20-17^T	*P. khanii* subsp. *guanajuatensis*	*H. atacamensis*	MN714283	PUJY01	PUJY01	Mexico	Machado *et al.* (2019)

TTO1^{T}	*P. laumondii* subsp. *laumondi*	*H. bacteriophora*	MK039077	WSFH01	WSFH01	Australia	Machado *et al.* (2018)
BOJ47^{T}	*P. laumondii* subsp. *clarkei*	*H. bacteriophora*	MK039078	NSCI01	NSCI01	Iran	Machado *et al.* (2018)
FRG04^{T}	*P. akhurstii* subsp. *akhurstii*	*H. indica*	MK039086	RCWE01	RCWE01	Australia	Machado *et al.* (2021) a
H3^{T}	*P. akhurstii* subsp. *bharatensis*	*Heterorhabditis* sp.	JX221722	PUWU01	PUWU01	India	Machado *et al.* (2021) a
SF41^{T}	*P. heterorhabditis* subsp. *heterorhabditis*	*H. zealandica*	MK039068	RCWA01	RCWA01	South Africa	Machado *et al.* (2018, 2021b)
Q614^{T}	*P. heterorhabditis* subsp. *aluminescens*	*Heterorhabditis* sp.	MN714272	JABBCS01	JABBCS01	Australia	Machado *et al.* (2018, 2021b)
9802892^{T}	*P. australis* subsp. *australis*	Clinical specimen	AY217761	JONO01	JONO01	Australia	Machado *et al.* (2021) b
PB68.1^{T}	*P. australis* subsp. *thailandensis*	*H. indica*	MT355494	LOMY01	LOMY01	Thailand	Machado *et al.* (2021) b
AM7^{T}	*P. noenieputensis*	*H. noenieputensis*	MK039084	JQ424881	JQ424884	South Africa	Machado *et al.* (2018)
C8404^{T}	*P. hainanensis*	*Heterorhabditis* sp.	MK039085	RCWD01	RCWD01	China	Machado *et al.* (2018)
PB45.5 ^{T}	*P. namnaonensis*	*H. baujardi*	MK039087	LOIC01	LOIC01	Thailand	Glaeser *et al.* (2018)
HG29 ^{T}	*P. caribbeanensis*	*H. bacteriophora*	MK039083	RCWB01	RCWB01	Guadeloupe	Machado *et al.* (2018)
H1^{T}	*P. hindustanensis*	*Heterorhabditis* sp.	JX221722	PUWT01	PUWT01	India	Machado *et al.* (2021) a
DSM 23513^{T}	*P. kleinii*	*H. georgiana*	MK039079	JAJAFY01	JAJAFY01	North America	Machado *et al.* (2018)
DSM 15194 ^{T}	*P. kayaii*	*H. bacteriophora*	MK039081	JAJAFZ01	JAJAFZ01	Turkey	Machado *et al.* (2018)
LJ24-63^{T}	*P. bodei*	*H. beicherriana*	MK039080	NSCM01	NSCM01	China	Machado *et al.* (2018)
BA1^{T}	*P. aegyptia*	*H. indica*	JAGJDU01	JFGV01	JFGV01	Egypt	Ghazal *et al.* (2014)
39-8^{T}	*P. thracensis*	*H. bacteriophora*	CP011104	CP011104	JAGJJU01	Turkey	Kwak and Shin (2015)
XINach ^{T} M1021	*P. temperata*	*H. megidis*	MK039073	JAJAFX01	AUXQ01	Russia	Machado *et al.* (2018) Park *et al.* (2013)

DSM 23271 ^{T}	*P. stackebrandtii*	*H. bacteriophora*	MK039075	PUJV01	PUJV01	North America	Machado *et al.* (2018)
T327 ^{T}	*P. tasmaniensis*	*H. zealandica*	MK039072	PUJU01	PUJU01	Australia	Machado *et al.* (2018)
UCH-936^{T}	*P. antumapuensis*	*H. atacamensis*	MZ676562	JAHZMK01	JAHZMK01	Chile	Castaneda-Alvarez *et al.* (2022)
3107 ^{T}	*P. cinerea*	*H. downesi*	MK039069	PUJW01	PUJW01	Hungary	Machado *et al.* (2018)
3265-86^{T}	*P. asymbiotica*	Clinical specimen	MK039071	RBLJ01	RBLJ01	USA	Machado *et al.* (2018)
APURE^{T}	*P. aballayi*	*H. amazonensis*	OP735347	JAPFCD01	JAPFCD01	Switzerland	Machado *et al.* (2023)
VN01 ^{T}	*X. vietnamensis*	*S. sangi*	NR115713	FJ823401	EU934514	Vietnam	Tailliez *et al.* (2006, 2010)
DSM16522 ^{T}	*X. japonica*	*S. kushidai*	DQ202310	FJ823400	EU934513	Japan	Tailliez *et al.* (2006, 2010)
ID10 ^{T}	*X. griffiniae*	*S. hermaphroditum*	DQ211710	FJ823399	EU934525	Indonesia	Tailliez *et al.* (2006, 2010)
DSM16337 ^{T}	*X. ehlersii*	*S. serratum*	AJ810294	FJ823398	EU934524	China	Lengyel *et al.* (2005); Tailliez *et al.* (2010)
FRM16 ^{T}	*X. doucetiae*	*S. diaprepesi*	DQ211709	FJ823402	EU934526	Martinique	Tailliez *et al.* (2006, 2010)
PR06-A ^{T}	*X. romanii*	*S. puertoricense*	DQ211717	FJ823403	EU934515	Puerto Rico	Tailliez *et al.* (2006, 2010)
SaV ^{T}	*X. kozodoii*	*S. arenarium*	DQ211716	FJ823404	EU934522	Russia	Tailliez *et al.* (2006, 2010)
G6 ^{T}	*X. poinarii*	*S. glaseri*	D78010	FJ823409	EU934543	USA	Tailliez *et al.* (2006, 2010)
SF87 ^{T}	*X. khoisanae*	*S. khoisanae*	JX623972	AB685736	AB685735	South Africa	Ferreira *et al.* (2013) b
Q1 ^{T}	*X. miraniensis*	*Steinernema sp.*	DQ211713	FJ823414	EU934520	Australia	Tailliez *et al.* (2006, 2010)
Q58 ^{T}	*X. beddingii*	*Steinernema sp.*	AY278675	FJ823415	EU934516	Australia	Marokhazi *et al.* (2003): Tailliez *et al.* (2010)
KE01 ^{T}	*X. hominickii*	*S. karii*	DQ211719	FJ823410	EU934517	Kenya	Tailliez *et al.* (2006, 2010)
USNJ01 ^{T}	*X. koppenhoeferi*	*S. scarabaei*	DQ205450	FJ823413	EU934532	USA	Tailliez *et al.* (2006, 2010)
ATCC19061 ^{T}	*X. nematophila*	*S. carpocapsae*	AY278674	FN667742	FN667742	USA	Tailliez *et al.* (2010); Chaston *et al.* (2011)

DSM16338 ^{T}	*X. szentirmaii*	*S. rarum*	AJ810295	FJ823416	EU934534	Argentina	Lengyel *et al.* (2005); Tailliez *et al.* (2010)
VC01 ^{T}	*X. mauleonii*	Unknown	DQ211715	FJ823417	EU934533	St. Vincent	Tailliez *et al.* (2006, 2010)
VP-2016b	*X. stockiae*	*S. surkhetense*	-	KX826948	KX826949	India	Bhat *et al.* (2017)
TH01 ^{T}	*X. stockiae*	*S. siamkayai*	DQ202309	FJ823425	EU934542	Thailand	Tailliez *et al.* (2006, 2010)
DSM16336 ^{T}	*X. innexi*	*S. scapterisci*	AJ810292	FJ823423	EU934540	Uruguay	Lengyel *et al.* (2005); Tailliez *et al.* (2010, 2012)
DSM16342 ^{T}	*X. budapestensis*	*S. bicornitum*	AJ810293	FJ823418	EU934535	Serbia	Lengyel *et al.* (2005); Tailliez *et al.* (2010)
IMI397775 ^{T}	*X. magdalenensis*	*S. australe*	NR109326	FJ798401	JF798402	Chile	Tailliez *et al.* (2012)
DSM17382 ^{T}	*X. indica*	*S. thermophilum*	AM040494	FJ823421	EU934538	India	Somvanshi *et al.* (2006); Tailliez *et al.* (2006, 2010)
USTX62 ^{T}	*X. cabanillasii*	*S. riobrave*	AY521244	FJ823422	EU934537	USA	Tailliez *et al.* (2006, 2010)
T228 ^{T}	*X. bovienii* subsp. *bovienii*	*S. feltiae*	X82252	JANAIF01	JANAIF01	Australia	Tailliez *et al.* (2010); Machado *et al*. (2023b)
XENO-1^{T}	*X. bovienii* subsp. *africana*	*S. africanum*	JAMGSK01	JAMGSK01	JAMGSK01	Africa	Machado *et al*. (2023b)
VLS^{T}	*X. lircayensis*	*S. unicornum*	MT466535	JACOII01	JACOII01	Chile	Castaneda-Alvarez *et al.* (2021)
30TX1^{T}	*X. thuongxuanensis*	*S. sangi*	KX602193	KX602194	KY451961	Vietnam	Kampfer *et al.* (2017)
DL20^{T}	*X. eapokensis*	*S. eapokense*	KX602187	KX602188	KY451960	Vietnam	Kampfer *et al.* (2017)
GDh7^{T}	*X. ishibashii*	*S. aciari*	AB243427	AB630947	AB630948	Japan	Kuwata *et al.* (2012)
K-12	*Escherichia coli*		NC000913.3	NC000913	NC000913		Gen Bank

3.3. Screening of insecticidal activity of Xenorhabdus and Photorhabdus against G. melonella

The graphical representation of the bacterial isolates showed insecticidal activity against *G. melonella* larvae was shown in Fig 8. Within 24 hr incubation, TS isolate showed the highest mortality rate (33.3%) while the lowest mortality rate was observed with RF isolate (26%). All the isolates showed mortality rate of 53.3 – 66.6% after 48 hr incubation. However, after 72 hr observation, the mortality rate of TD and RF isolates was 90%, TS isolate being the highest with 96% and PTS with 86.6% which is the lowest. All the isolates caused 100% mortality of the larvae after 96 hr incubation with bacterial suspension.

Infection of the larvae with *Xenorhabdus* isolates (RF and PTS) showed yellowish to brownish colouration while infection with *Photorhabdus* (TS and TD) isolates showed brick red colouration. The difference in the colour of the dead larvae were also observed in the case of infection of the larvae with *Steinernema* and *Heterorhabditis*

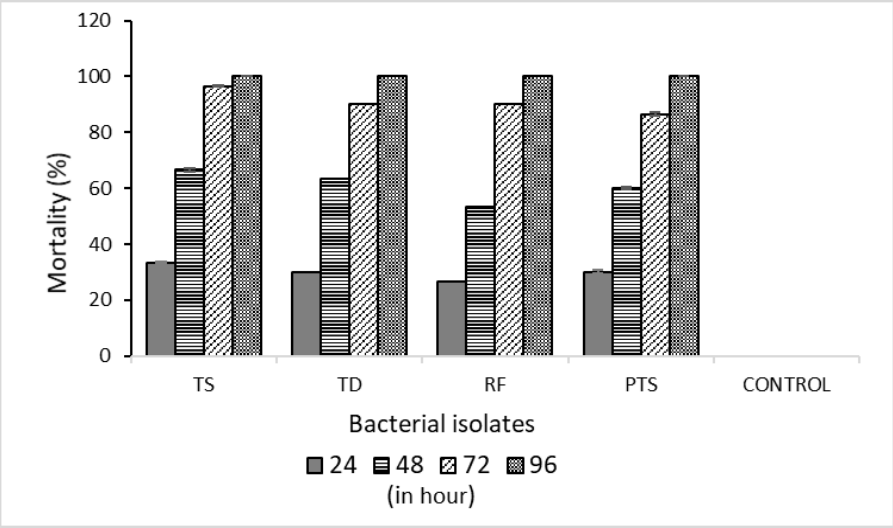

Figure 13: Activity of cell suspensions of bacterial isolates against *Galleria mellonella* within 96 hours observation. Results were expressed as mean ± SE.

Figure 14: Treatment of *Galleria mellonella* with the isolates. (*Xenorhabdus* and *Photorhabdus*)

(A) Control (*G. melonella* larvae treated with ditilled water)
(B) *G. melonella* larvae infected with *Xenorhabdus* isolates
(C) *G. melonella* larvae infected with *Photorhabdus* iaolates

3.4. Antimicrobial activity.

The ethyl acetate extracts of all the bacterial isolates inhibited the growth of *E. coli* (ATCC 10536), *K. pneumoniae* (ATCC 10031), *P. aeruginosa* (ATCC 10145), and *B. subtilis* (ATCC 11774) within 24 h of incubation. (Figure 3). Additionally, from our study, the extracts of the bacterial isolates showed significant variations of growth inhibition against the selected pathogenic bacteria ($df=3,43$; $F=48.56$; $p<0.05$). Among the studied bacterial isolates, the extracts of TS and TD were found to be most potent against the pathogenic bacteria with a growth inhibition zone of 13.67– 16.33 mm diameter. The highest inhibition on the pathogenic bacteria was recorded with *K. pneumoniae* (ATCC 10031) where the bacterial extract of *Photorhabdus* isolates (TS) provided a clear inhibition zone of 16.33 ± 0.33 mm in diameter. In addition, the bacterial extract of *X. vietnamensis* inhibited the growth of all the four tested bacterial strains, with a clear inhibition zone of 13 – 13.67 mm diameter. The extract of *X. stockiae* showed comparatively lower activity against the pathogenic bacteria as compared to the other isolates, exhibiting a clear inhibition zone of 10 – 11.67 mm diameter against the tested bacterial strains. The Mean ± SEM of inhibitory activity of the bacterial isolates against pathogenic bacteria was given in Table 2.

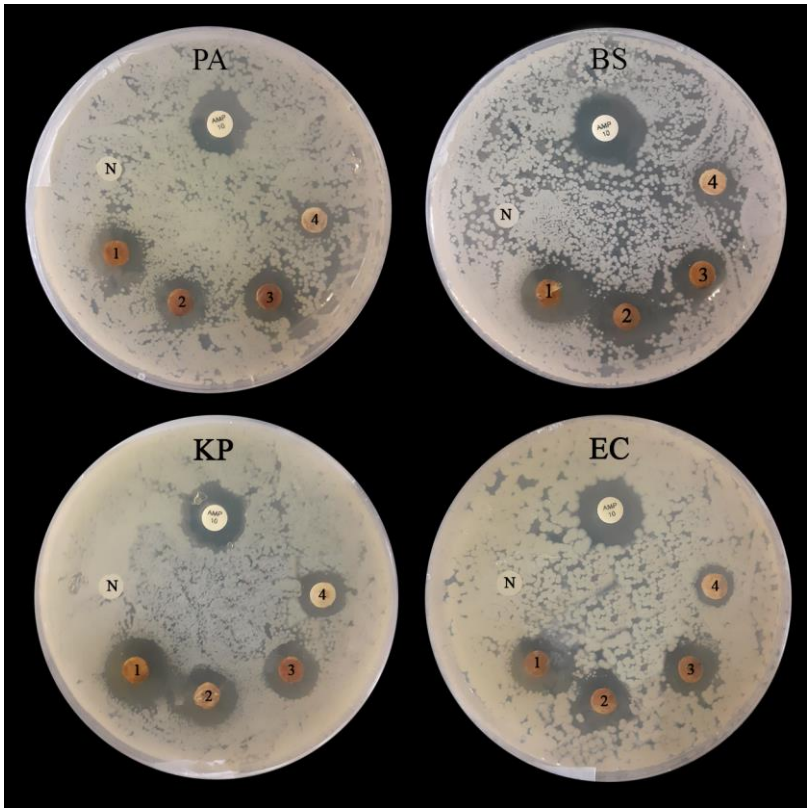

Figure 15. Antibacterial activities of bacterial isolates against pathogenic bacteria using disk diffusion method. 1=TS, 2=TD, 3=RF, 4=PTS, AMP=Ampicillin standard disk (10 µg) and N=Negative control (DMSO).

Table 4. Activity of bacterial extracts against pathogenic bacteria using disk diffusion method (Mean±SEM)

Bacterial strain	*E. coli* (ATCC 10536)	*K. pneumoniae* (ATCC 10031)	*P. aeruginosa* (ATCC 10145)	*B. subtilis* (ATCC11774)
TS	15.33±0.33	16.33±0.33	14.33±0.33	13.67±0.33
TD	14±0.57	14.33±0.33	13.67±0.67	13.67±0.33
RF	13.33±0.33	13.67±0.33	13±0.57	13.33±0.33
PTS	10±0.57	11.67±0.33	10±0.57	10.67±0.33

3.4.1. Minimum Inhibitory Concentration and Minimum Bactericidal Concentration (MIC and MBC).

The MIC and MBC values of *Photorhabdus* and *Xenorhabdus* extracts against the pathogenic bacteria were given in Table 3. The MIC value of both *Photorhabdus* isolates ranges from 3.90 – 1.95 mg/ml and the MBC value range from 7.81–1.95 mg/ml. Furthermore, the *Photorhabdus* isolates, TS and TD exhibited the highest activity at the same MIC and MBC value (1.95 mg/ml) against *P. aeruginosa* (ATCC 10145) and *K. pneumoniae* (ATCC 10031), respectively. In addition, the lowest inhibitory activity was observed with *Photorhabdus* isolates (TS and TD) against *B. subtilis* at the MIC and MBC values of 3.90 mg/ml and 7.81 mg/ml, respectively.

In the case of *Xenorhabdus* isolates, both *X. vietnamensis* and *X. stockiae* show MIC and MBC values ranging from 7.81–3.90 mg/ml and 15.62 – 3.90 mg/ml, respectively. The highest activity of *Xenorhabdus* isolates was observed at the same value of MIC and MBC (3.90 mg/ml) with *X. vietnamensis* and *X. stockiae* against *E. coli* (ATCC 10536) and *K. pneumoniae* (ATCC 10031), respectively. In addition, both the *Xenorhabdus* isolates show the lowest activity against *B. subtilis* (ATCC 11774) with a MIC value of 7.81 mg/ml and an MBC value of 15. 62 mg/ml. Therefore, based on the observed MIC and MBC values, the overall activities of *Photorhabdus* extracts were higher against the test pathogenic organisms as compared to *Xenorhabdus* isolates.

Table 5. MIC and MBC of bacterial extracts against pathogenic bacteria (mg ml^{-1})

Bacterial strain	*E. coli* (ATCC 10536)		*K. pneumoniae* (ATCC 10031)		*P. aeruginosa* (ATCC 10145)		*B. subtilis* (ATCC 11774)	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
TS	3.90	3.90	1.95	3.90	1.95	1.95	3.90	7.81
TD	1.95	3.90	1.95	1.95	3.90	3.90	3.90	7.81
RF	3.90	3.90	3.90	7.81	3.90	7.81	7.81	15.62
PTS	7.81	7.81	3.90	3.90	3.90	7.81	7.81	15.62

3.5. Screening of antifungal activity of Xenorhabdus and Photorhabdus against F. oxysporum and *F. solani*:

The inhibition percentage of bacterial isolates over *F. oxysporum* is depicted in Fig. 8, 9 and 10. The growth inhibition of *F. oxysporum* was observed 48 hr post–incubation. Among the isolates, *Photorhabdus* (TD and TS) caused 50–60% inhibition after 48 hr that increased to 76–79% inhibition after 96 hr of incubation. A further rise in the antifungal activity showed 84–87% inhibition after 192 hr of incubation. In the case of *Xenorhabdus* isolates, the percent inhibition of growth increased 48 hr to 96 hr post-incubation, followed by decrease in inhibition of the fungal growth after 192 hr of incubation. After 48 hr post-inoculation, *X. surkhetense* inhibited *F. oxysporum* upto 66% with a further rise of 75% after 96 hr of incubation. *X. vietnamensis* inhibited 67 % and 76% fungal growth after 48 and 96 hr of incubation, respectively. The study showed that all the bacterial isolates were highly effective against *F. oxysporum* with significant growth inhibition at different time ($p \leq 0.05$). However, no significant difference in antifungal activity was observed among the isolates ($p \geq 0.05$).

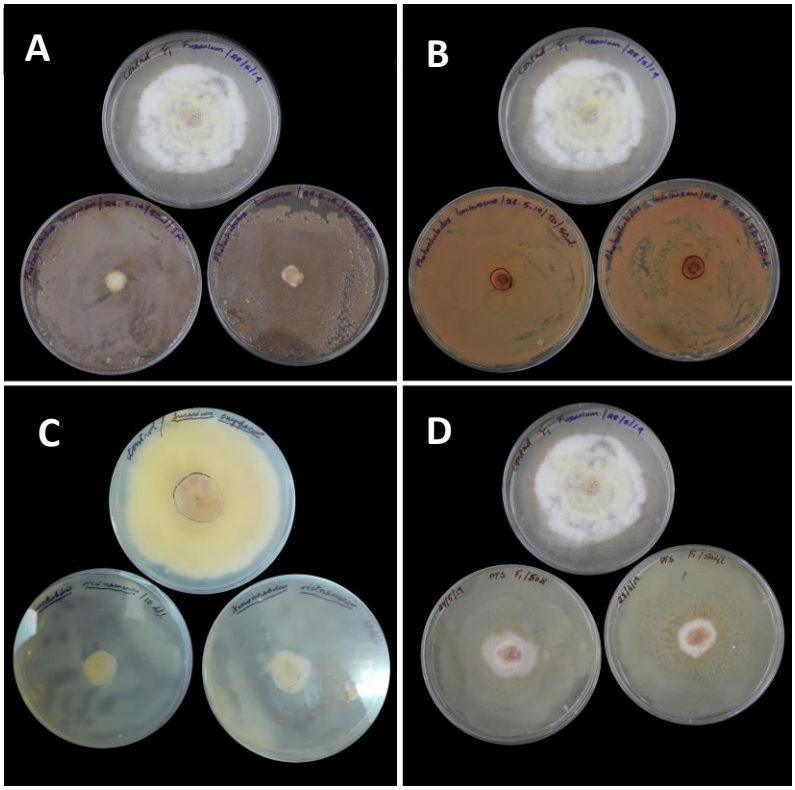

Figure 16. Antifungal activity of isolates against *Fusarium oxysporum* (A) Activity of *P. namnaonensis* TD (B) Activity of *P. hindustanensis* TS (C) Activity of *X. vietnamensis* RF (D) Activity of *X. stockiae* PTS

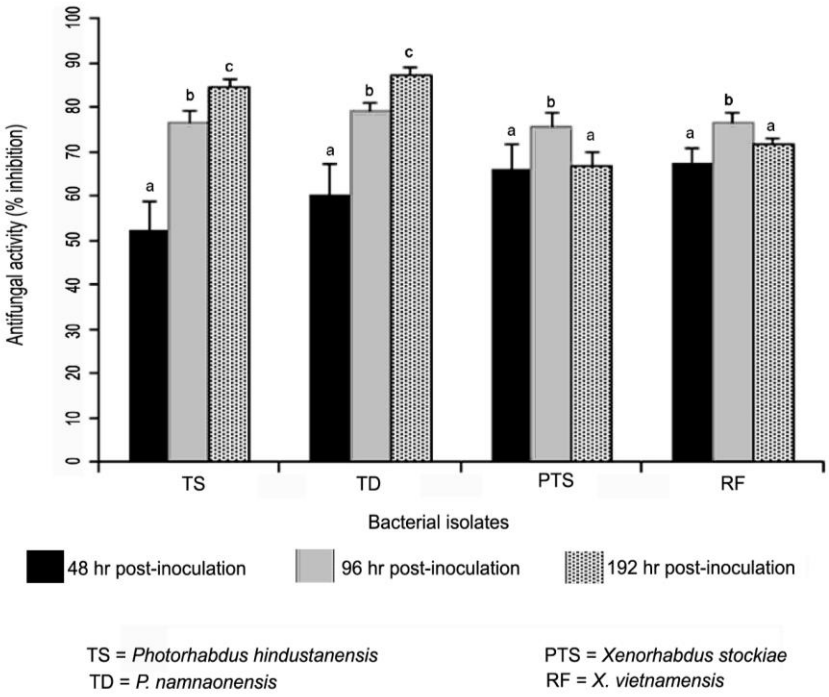

Figure 17: Antifungal activity of the bacterial isolates against *F. oxysporum*. The results were presented as growth inhibition (%) ± SEM. For all the isolates, bars with different lowercase letter stand for significant differences (P ≤ 0.05) of inhibition at different incubation time (hr).

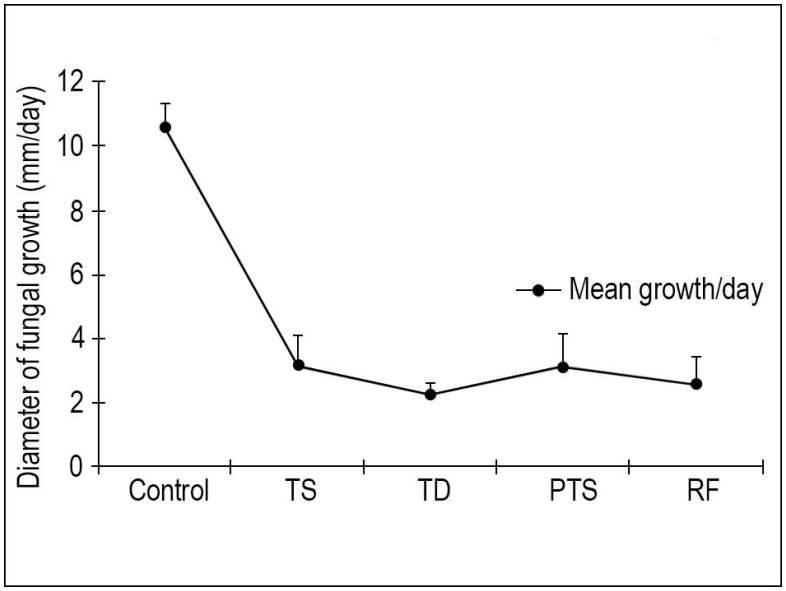

Figure 18: Effects of bacterial isolates on the growth of *F. oxysporum* in comparision to the control plate. The growth rate of the fungus per day is presented in the form of Mean ± SEM

The bacterial isolates showed inhibition of growth against *F. solani* within 196 hr post inoculation. In case of TS isolate, a rise in percent inhibition (36.14 – 49.65%) was observed within the observation period. However, TD isolate caused increased inhibition of *F. solani* from 50.22 – 52% within 96 hr post inoculation with a slight increased to 52.17% within 192 post inoculation. RF isolate showed significant increase of growth inhibition from 47.98 – 64.67% within 96 hr post inoculation which further rise to 76.54 % at 192 hr post inoculation. The increase in inhibition

percentage was observed on PTS isolate with 66.04% after 48 hr post inoculation which further increase to 71.33% and 75.85% within 96 and 192 hr respectively. The study showed that the bacterial isolates were highly effective against *F. solani*. The significant growth inhibition was observed against the activity of TS, RF and PTS at different time ($p \leq 0.05$). However, TD isolate showed no significant inhibition activity against *F. solani* at different time ($p < 0.05$).

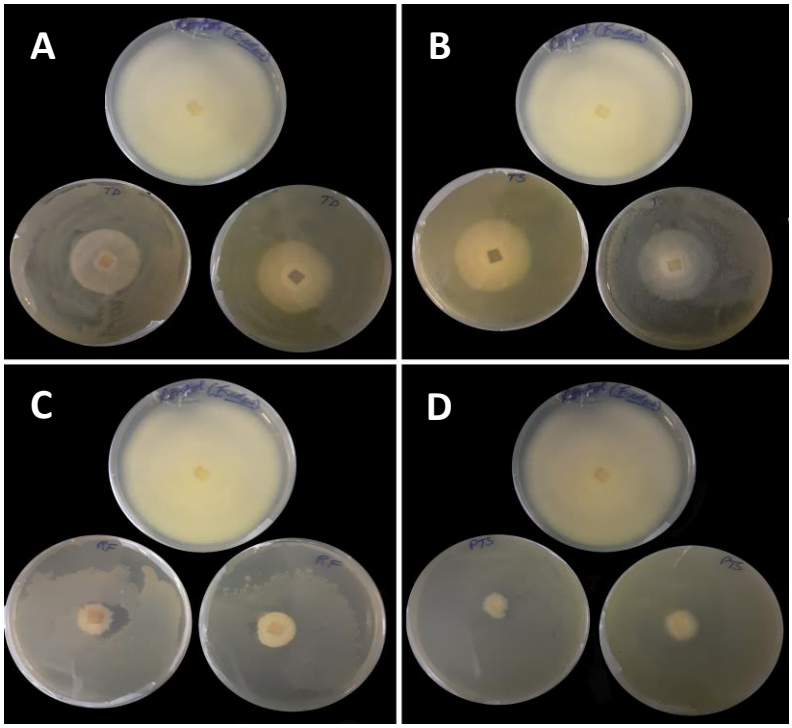

Figure 19. Antifungal activity of isolates against *Fusarium solani* (A) Activity of *P. namnaonensis* TD (B) Activity of *P. hindustanensis* TS. (C) Activity of *X. stockiae* PTS. (D) Activity of *X. vietnamensis* RF.

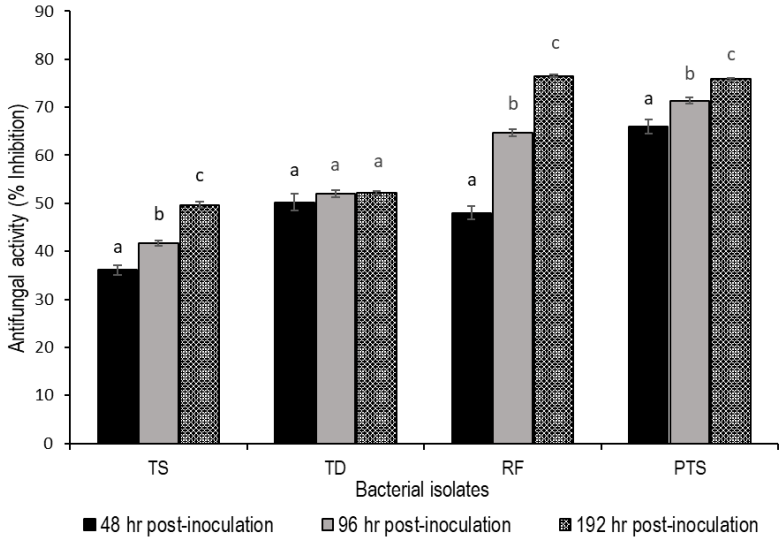

Figure 20: Antifungal activity of the bacterial isolates against *F. solani*. The results were presented as growth inhibition (%) ± SEM. For all the isolates, bars with different lowercase letter stand for significant differences (P ≤ 0.05) of inhibition at different incubation time (hr).

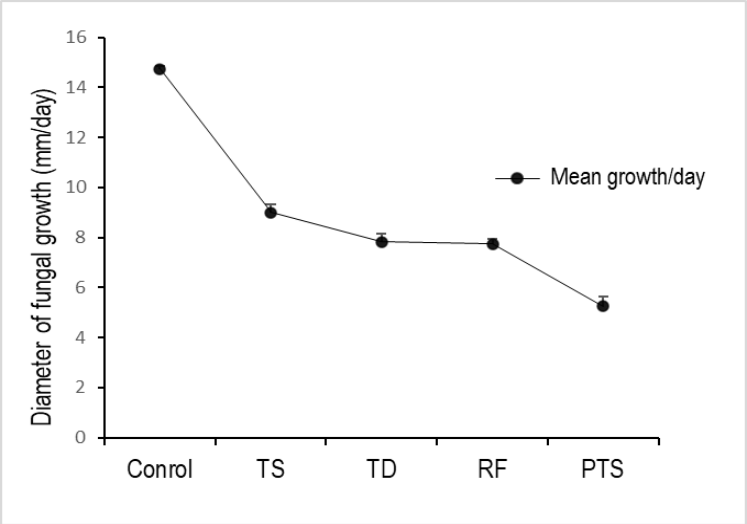

Figure 21: Effects of bacterial isolates on the growth of *F. solani* in comparision to the control plate. The growth rate of the fungus per day is presented in the form of Mean ± SEM

The bacterial isolates showed inhibition of growth against *C. albicans* within 196 hr post inoculation. In case of TS isolate, a rise in percent inhibition (69.39-93.24%) was observed within the observation period.TD isolate caused increased inhibition of *C. albicans* from 51.08 – 88.51%. RF isolate showed significant increase of growth inhibition from 68.47 – 93.46% within 192 hr post inoculation. The increase in inhibition percentage was observed on PTS isolate with 66.04% after 48 hr post inoculation which further increase to 54.85% and 79.05% within 96 and 192 hr respectively. The study showed that the bacterial isolates were highly effective against *C. albicans*. The significant growth inhibition was observed against the activity of TS, RF and PTS at different time (p ≤ 0.05).

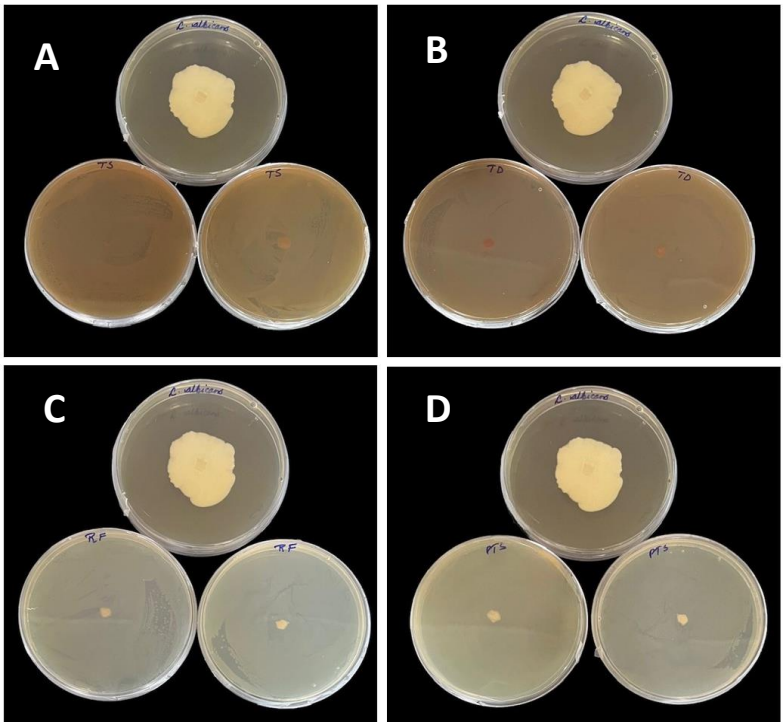

Figure 22. Antifungal activity of isolates against *Candica albicans* (A) Activity of *P. hindustanensis* TS (B) Activity of *P. namnaonensis* TD. (C) Activity of *X. stockiae* PTS. (D) Activity of *X. vietnamensis* RF

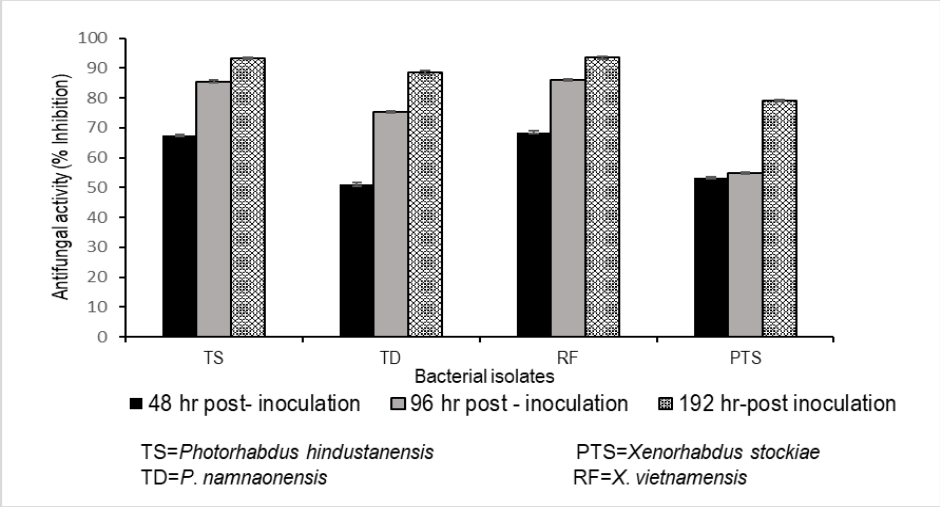

Figure 23: Antifungal activity of the bacterial isolates against *C. albicans*. The results were presented as growth inhibition (%) ± SEM. For all the isolates, bars with different lowercase letter stand for significant differences ($P \leq 0.05$) of inhibition at different incubation time (hr).

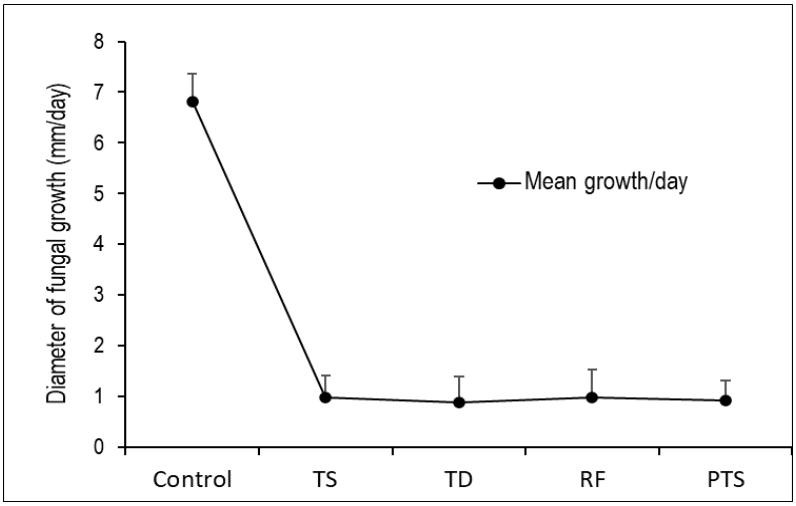

Figure 24: Effects of bacterial isolates on the growth of *C. albicans* in comparision to the control plate. The growth rate of the fungus per day is presented in the form of Mean ± SEM

4. OVERALL ACHIEVEMENTS

- The present study emphasizes the occurrence and identification of symbiotic bacteria from Mizoram, North-east India, an Indo-Burma biodiversity hotspot region and provides information regarding the capability of their activity towards various pathogenic bacteria.

- Molecular characterization using the selected primer sequences revealed that *P. luminescens* subsp. *namnaonensis* was first reported in India. All the developed sequences were submitted to NCBI GenBank for documentation and future references.

- Our experimental results highlight the potential antifungal activity of the isolates to a certain degree and its delivery methods in the field conditions needs to be investigated either through formulations with other fungicides or the secondary products of the isolates alone.

- Variations of activities including degree of inhibition against the tested pathogenic bacteria were observed among the isolates. This may be attributed to several factors such as the production of secondary metabolites by *Photorhabdus* and *Xenorhabdus*, including media used for culture, medium pH, temperature, inoculation volume, fermentation time, rotary speed and bacterium – nematode affected insect cadaver conditions

- The activity of these isolated organisms against insect pest provides information regarding the potential of the isolates and further formulation and detail analysis needs to be carried on to make it available for field applications.

- However, detailed analysis and studies need to be carried on to increase the knowledge of the exact genetic information, composition of chemical compounds, and mode of action against other microbes since these extracted metabolites could be a promising antibiotic in the future.

- Since the study area is a part of the world biodiversity hotspot region, the organisms distributed in the region are distinct with different degree of activities. Therefore, it is recommended to cover more areas for documentation of these kinds of microorganisms.

- The information provided in this study will pave the way for identification and further analysis of certain microorganisms and their metabolites for combating various diseases and insect pests worldwide.

5 IMPACTS OF FELLOWSHIP IN IHR

1.1 Socio-Economic Development (max. 500 words, in bullet points)
1.2 Scientific Management of Natural Resources In IHR (max. 500 words, in bullet points)
1.3 Conservation of Biodiversity in IHR (max. 500 words, in bullet points)
1.4 Protection of Environment (max. 500 words, in bullet points)
1.5 Developing Mountain Infrastructures (max. 500 words, in bullet points)
1.6 Strengthening Networking in IHR (max. 700 words, in bullet points)

6. EXIT STRATEGY AND SUSTAINABILITY

Morphological and molecular characterization of the symbiotic bacteria is necessary to provide documentation of endemic species and the novel species/strains of the region rendering effective use of the bacteria and its host as biological control and conservation programmes. Introducing EPB as a component of Integrated Pest management programme in Mizoram and selection of high virulence, persistence, reproducing capacity etc. species/strains for a good biological control agent. Introducing microbial products as an alternate agent to classical antibiotics for combating pathogenic organisms will pave a way for developing novel antibiotics for future approach.

Another beneficial microbe with a high potential of antibacterial activity was also evaluated during the fellowship period. Article was made available and will be communicated in a reputed journal.

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8 ACKNOWLEDGEMENTS

This work was funded by National Mission on Himalayan Studies (NMHS), GBPNIHESD under Himalayan Fellowship (U/ I ID: HSF 2018-19/I-25/03; Sanction No. GBPNI/NMHS-2018-19/HSF 25-03/154, Dt. 17.12.2018. We are thankful to the Principal, Pachhunga University College; Head, Department of Zoology, Pachhunga University College for providing the necessary research facilities to carry out this work.

9 APPENDICES

1. Lalramchuani, M., H.C. Lalramnghaki, R. Vanlalsangi, E. Lalhmingliani, Vanramliana and Lalramliana: Characterization and screening of antifungal activity of bacteria associated with entomopathogenic nematodes from Mizoram, North-Eastern India. J. Environ. Biol., 41, 942-950 (2020). http://doi.org/10.22438/jeb/4(SI)/MS_1913
2. Lalramchuani, M., Lalramnghaki, H. C., & Lalhmingliani, E: Molecular Characterization and Antibacterial Activities of *Photorhabdus* and *Xenorhabdus* from Mizoram, North-East India. J Pure Appl Microbiol., *17*, 1560 - 1577 (2023) https://doi.org/10.22207/JPAM.17.3.18
3. Oral presentation at 'International Conference on Recent Advances in Animal Sciences'-2019 Organised by Dept of Zoology, Pachhunga University College, from 6-8 November, 2019. on the topic- **'Evaluation of the larvicidal activity of two locally isolated Entomopathogenic Bacteria (*Xenorhabdus* and *Photorhabdus*) against *Galleria mellonella* L'.**
4. Poster Presentation at 'TROPACON N.E 2022 organised by Indian Academy of Tropical Parasitology, NE Chapter and Zoram Medical College, from 9-10 November, 2022 on the topic '**Antibacterial activity of *Photorhabdus* and *Xenorhabdus* isolated from Mizoram, North-East India'.**
5. Poster presentation at 'International Conference on Biodiversity and Conservation (ICBC) organized by Dept of Zoology and Fishery Science, St. Anthonys College, Shillong, from 14-15 December 2022 on the topic '**Antagonistic Potential of *Photorhabdus* and *Xenorhabdus* against *F. solani* from Mizoram, North- East India'**

******

(Signature of HJRF)

(NMHS FELLOWSHIP COORDINATOR)

(HEAD OF THE INSTITUTION)

Principal
Pachhunga University College
Aizawl : Mizoram

Place: Aizawl
Date: 28/11/2022

PACHHUNGA UNIVERSITY COLLEGE AIZAWL

For the Period: 17/12/2018 to 16/12/2021

1.	Title of the fellowship/Scheme:	**NMHS Research Fellowship**
2.	Name of the Principal Investigator & Organization:	**Prof. Lalramliana**
3.	NMHS-PMU, G.B. Pant National Institute of Himalayan Environment, Kosi-Katarmal, Almora, Uttarakhand Letter No. and Sanction Date of the Fellowship:	GBPNI/NMHS-2018-19/HSF25-03 Dt. 17. 12. 2018
4.	Amount received from NMHS-PMU, G.B. Pant National Institute of Himalayan Environment, Kosi-Katarmal, Almora, Uttarakhand during the fellowship period (Please give number and dates of Sanction Letter showing the amount paid):	**35,12,518**
5.	Total amount that was available for expenditure (including commitments) incurred during the fellowship period:	**35,12,518**
6.	Actual expenditure (excluding commitments) incurred during the fellowship period:	**34,28,049**
7.	Unspent Balance amount refunded, if any (Please give details of Cheque no. etc.):	
8.	Balance amount available at the end of the fellowships:	**99,718**
9.	Balance Amount:	**99,718**
10.	Accrued bank Interest:	**15,249**

Certified that the expenditure of **Rs. 34,28,049 (Thirty-four lakhs twenty-eight thousand and forty-nine only)** mentioned against Sr. No. 6 was actually incurred on the fellowship/scheme for the purpose it was sanctioned.
Date:21/12/22

(Signature of Principal Investigator)

Professor
Department of Zoology
Pachhunga University College
Mizoram University

(Signature of Registrar/Finance Officer)

Section Officer (Fin)
Pachhunga University College
Aizawl : Mizoram

(Signature of Head of the Institution)

Principal
Pachhunga University College
Aizawl : Mizoram

PACHHUNGA UNIVERSITY COLLEGE ★ AIZAWL ★

FOR AKAS & ASSOCIATES LLP
Chartered Accountants
Partner
M. No. 98017
21/12/22

Unique Document Identification Number (UDIN) for this document is 22098017BFWDOD3892

Date:

COMPETENT AUTHORITY
NATIONAL MISSION ON HIMALAYAN STUDIES (GBP NIHE)

Statement of Consolidated Expenditure

[Pachhunga University College]

Statement showing the expenditure of the period from **17/12/2018 to 16/12/2021**
Sanction No. and Date: **GBPNI/NMHS-2018-19/HSF25-03/154/488** **Date: 17.12.2018**

1. Total outlay of the Fellowship: **Rs. 36,41,616 (Thirty-Six Lakh Forty-One Thousand Six Hundred Sixteen)**

2. Date of Start of the Fellowship : **17/12/2018**

3. Duration : **03 yrs**

4. Date of Completion : **16/12/2021**

a) Amount received during the fellowship period : **35,12,518**

b) Total amount available for Expenditure : **35,12,518**

S. No.	Budget head	Amount received	Expenditure	Amount Balance/ excess expenditure
1	Salaries	23,22,756	22,23,720	99,036
2.	Permanent Equipment purchased	Nil	Nil	Nil
3	Contingency (including domestic travel)	8,70,000	8,69,318	682
4.	Expendables/Consumables	Nil	Nil	Nil
5	Institutional charges	3,19,762	3,19,762	0
7	Accrued bank Interest	15,249	**15,249***	0
7	**Total**	**35,27,767**	**34,28,049**	**99,718**

Certified that the expenditure of **Rs. 34,28,049 (Thirty-four lakhs twenty-eight thousand and forty-nine only)** mentioned against Sr. No.12 was actually incurred on the fellowship/ scheme for the purpose it was sanctioned.
* Interest **Rs. 15,249** deposited back to the Govt.

Date:12/12/2022

(Signature of Principal Investigator)

Professor
Department of Zoology
Pachhunga University College
Aizawl : Mizoram

(Signature of Registrar/Finance Officer)

Section Officer (Fin)
Pachhunga University College
Aizawl : Mizoram

(Signature of Head of the Institution)

Principal
Pachhunga University College
Aizawl : Mizoram

For AKAS & ASSOCIATES LLP
Chartered Accountants
FRN: 022476N
Partner
AJAY
M. No. 98017
AIZAWL
Pl. No. 9801
21/12/22

PACHHUNGA UNIVERSITY COLLEGE
AIZAWL

Unique Document Identification Number (UDIN) for this document is 22098017BFWDOD3892

ACCEPTED AND COUNTERSIGNED

Date:

COMPETENT AUTHORITY
NATIONAL MISSION ON HIMALYAN STUDIES (GBP NIHE)

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PACHHUNGA UNIVERSITY COLLEGE

(A CONSTITUENT COLLEGE OF MIZORAM UNIVERSITY)

NAAC Accredited A+
Institution 45th NIRF
RANKING 2022

AIZAWL - 796001: MIZORAM, INDIA

This is to certify that Rs 15,249 (Fifteen thousand two hundred and forty-nine) has been earned as interest and deposited back to NMHS GIA General.

(HEAD OF THE INSTITUTION)

(Signed and Stamped)

Principal
Pachhunga University College
Aizawl : Mizoram

PACHHUNGA UNIVERSITY COLLEGE
AIZAWL

DIRECT BENEFIT TRANSFER (DBT) DETAILS

Scheme Name:	**National Mission on Himalayan Studies (NMHS)**
Scheme Type:	**Central Sector (CS) Grant-in-Aid Scheme**
Scheme Code:	**NMHS**
Category:	**Fellowship Grant**
Month-Year:	**December 2018-December 2021**

PRO FORMA FOR DBT DETAILS

University/Institution Name: PACHHUNGA UNIVERSITY COLLEGE, AIZAWL, MIZORAM

S. No.	Position (H-RA, H-JRF/ H-JPF)	Name	DoB*	DoJ*	PI	Research title	Objectives	Study Area, IHR State	Contact details (Complete corresponding address), Mobile No., E-mail ID	Bank details (Account number, IFSC Code)	Emoluments/Fellowship
	H-RA	Dr. C. Malsawmtluangi	01/03/1979	17/12/2018	Dr. Lalramliana	Diversity and characterization of helminth parasites of freshwater fishes and evaluation of their zoonotic potential in Mizoram, northeast India	• Explore diversity and identify the helminth parasites of common freshwater fishes from various localities in Mizoram • Morphological and molecular characterization (using ITS and COI region) of the parasites species • Assess the role of fishes in disseminating plausible helminthes in human and other animals.	Mizoram	Research Centre, Pachhunga University College, Aizawl, 796005 Mob:8257996079 Email: mschenkual@gmail.com	Ac:30812792852 IFSC: SBIN0007058	Rs. 13,21,920

2.	H-JRF	Mary Lalramchuani	21/04/1993	17/12/2018	Dr. Lalramliana	Diversity and sustainable utilization of the entomopathogenic bacteria (*Xenorhabdus* and *Photorhabdus*) from Mizoram, northeast India	• To isolate, identify and study the biochemical activity of entomopathogenic bacteria associated with Steinernema spp. and Heterorhabditis spp. of Mizoram, NE India. • To study the genetic diversity and molecular phylogeny of isolated bacteria using 16SrRNA, rec A and gyr B gene region. • To evaluate the insecticidal and antimicrobial activity of the isolated bacteria	Mizoram	Research Centre, Pachhunga University College, Aizawl, 796005 Mob: 9862334263 Email: marylalramchuani @gmail.com	Ac:30812792852 IFSC: SBIN0007058	Rs. 9,01,800

Note: For each month, the DBT Details Pro forma dully filled and signed for each Himalayan Fellowship Grant under NMHS must be submitted at finance.nmhspmu2017@gmail.com; nmhspmu2016@gmail.com. *DoB (Date of Birth); DoJ (Date of Joining).

(Authorized Signatory)
Principal
Pachhunga University College
Aizawl : Mizoram

December 2018-December 2021– Latest Updated List of Himalayan Researchers or Fellows *(working in the current time)*

S#	Name	Fellowship (RA/JRF/JPF)
1.	Dr. C. Malsawmtluangi	13,21,920 (RA)
2.	Mary Lalramchuani	9,01,800 (JRF)

Annexure-IV

Details and Declaration of Refund of Any Unspent Balance

Please provide the details of refund of any unspent balance as RTGS (Real-Time Gross System) in favor of **NMHS GIA General** and declaration on the official letterhead duly signed by the Head of the Institution.

Kindly note the further Bank A/c Details as follows:

Name of NMHS A/c: NMHS GIA General
Bank Name & Branch: Central Bank of India (CBI), Kosi Bazar, Almora, Uttarakhand 263643
IFSC Code: CBIN0281528
Account No.: 3530505520 (Saving A/c)

In case of any queries/ clarifications, please contact the NMHS-PMU at e-mail: nmhspmu2016@gmail.com

Annexure-V

Technology Transfer and/ or Intellectual Property Rights Certificate

With a view to encourage the institutions to file patent applications on their innovations, motivate them to transfer their technologies for commercialization, and facilitate them to reward their inventions, the following instructions are issued.

1. In these instructions:

(a) **"Institution"** means any technical, scientific or academic establishment where research work is carried out through funding by the Central / State Government.

(b) **"Intellectual Property Rights"** include patents, registered designs, copyrights and layout design of integrated circuits.

(c) **"Inventor"** means an employee of the institution whose duties involve carrying out of scientific or technical research.

2. **Scope:** These instructions apply to those institutions receiving funds for research projects/ fellowships from NMHS, the Ministry of Environment, Forest and Climate Change (MoEF&CC).

3. **Inventions by institutions:** Institutions shall be encouraged to seek protection of Intellectual Property Rights (IPR) to the results of research through R&D projects/ fellowships. While the patent may be taken in the name(s) of inventor(s), the institutions shall ensure that the patent is assigned to it & DBT, GOI. The institution shall take necessary steps for commercial exploitation of the patent on non-exclusive basis. The institution is permitted to retain the benefits and earnings arising out of the IPR. However, the institution may determine the share of the inventor(s) and other persons from such actual earnings. Such share(s) shall be limited to 1/3rd of the actual earnings.

4. **Inventions by institutions and industrial concerns:** IPR generated through joint research by institution(s) and industrial concern(s) through joint efforts can be owned jointly by them as may be mutually agreed to by them and accepted by the Department through a written agreement. The institution and industrial concern may transfer the technology to a third party for commercialization on exclusive/non-exclusive basis. The third party, exclusively licensed to market the innovation in India, must manufacture the product in India. The joint owners may share the benefits and earnings arising out of commercial exploitation of the IPR. The institution may determine the share of the inventor(s) and other persons from such actual earnings. Such share(s) shall not exceed 1/3rd of the actual earnings.

5. **Patent Facilitating Fund:** The institution shall set apart not less than 25 per cent of such earnings for crediting into a fund called Patent Facilitating Fund. This Fund shall be utilized by the institution for updating the innovation, for filing new patent applications, protecting their rights against infringements, for creating awareness and building competency on IPR and related issues.

6. **Information:** The institutions shall submit information relating to the details of the patents obtained, the benefits and earnings arising out of IPR and the turnover of the products periodically to the Department/Ministry, which has provided funds.

7. **Royalty-free license:** The Government shall have a royalty-free license for the use of the intellectual property for the purposes of the Government of India.

(HEAD OF THE INSTITUTION)

(Signed and Stamped)

Principal
Pachhunga University College
Aizawl : Mizoram