THE HELMINTH FAUNAL SPECTRUM OF RODENT HOSTS FROM BAMBOO GROWING AREAS IN MIZORAM, NORTHEAST INDIA

by

C. MALSAWMTLUANGI

DEPARTMENT OF ZOOLOGY

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN ZOOLOGY

of

NORTH-EASTERN HILL UNIVERSITY

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ABSTRACT

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December, 2010

I, **C. Malsawmtluangi**, hereby declare that the subject matter of the thesis is the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University / Institute.

This is being submitted to the North-Eastern Hill University for the degree of Doctor of Philosophy in Zoology.

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Dedicated to my loving mother

Zahmingthangi (Late)

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ACKNOWLEDGEMENT

It is with immense pleasure that I take this opportunity to thank all the people who have helped me all through my research work. First and foremost I give my praise and gratitude to the almighty God who has watched over me all through the years of my research.

My sincere and heartfelt gratitude goes to my teacher and guide Prof. (Mrs) V. Tandon, North-Eastern Hill University, Shillong for her able guidance, constant encouragement, support and patience throughout the course of my research. I couldn't have asked for anyone better.

I am extremely grateful to Prof. (Mrs) R. N. K. Horoo, the present Head of the Department, Department of Zoology, North-Eastern Hill University, Prof. B.B. P. Gupta and Prof. S. B. Prasad the ex-head, for allowing me to use the departmental facilities.

I sincerely acknowledge the Ministry of Environment and Forest Aicoptax project to Prof. V. Tandon for financial support without which these work would not have been possible. I would like to thank ICAR and Zoological Survey of India, Kolkata for helping me in idetification of the rodent hosts.

I would like to thank all my colleague in Parasitology lab Wapang, Pramod, Lalit, Sunila, Donald, Sudeep, Jollene, Voleentina, Ranjana for their constant support and

encouragement and not to forget Bahdeng (Judistar) and KongDohling for helping me in many ways.

I would like to thank all my friends in Nehu Saipari, Audrey, Puii and Sama who have helped me during my stay here in NEHU.

I would like to express my deep sense of gratitude to my Late mother who has always wanted me to continue my studies inspite of all odds. My father and my sister who has given me a constant moral support all throughout my studies.

I am deeply grateful and indebted to my husband who has allowed me to continue my study and for always being my strength and support all through these years, and my in laws who helped me in no small measures.

Place:

Dated:

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PREFACE

Rodents, one of the diverse groups of mammals characterized by their chisel-shaped incisors, are one of the most successful, abundant and destructive pests inflicting incalculable losses to standing crops, harvested crops in threshing floors, stored food grains and other commodities. Rodents also harbour a number of helminth parasites, which can be transmitted to human beings and other vertebrates, and also serve as reservoir host and aid in dissemination of these worms to domestic animals and man, thus causing zoonoses

In northeastern region of India, in the bamboo growing areas of the State of Mizoram in particular, it is widely believed that rodent outbreaks occur in consonance with the gregarious flowering of bamboo plants; the bamboo flowering which is a periodic phenomenon, is a bad omen and is considered a harbinger of famine. In Mizoram, the most abundant bamboo species is *Melocanna baccifera.* In this bamboo species, the flowering, locally known as 'Mautam', occurs periodically after every 48±1 years. The last major episode of 'Mautam' was recorded in the year 1959, when an explosion of rodent population and consequent occurrence of famine was also reported. According to the folk belief, the rodents feed on the large quantities of highly nutritious seeds of these dying bamboo trees, which, in turn, is believed to trigger a rapid increase in the birth rate of the rodents, leading to their population explosion. Following depletion of the bamboo seeds, the huge populations of rats attack fields of paddy and vegetables causing huge losses and even venture into human dwellings and attacking granaries etc. Thus, occurrence of famine in concurrence with the flowering phenomenon is a real happening and not merely a superstitious belief. Following its periodicity the next 'Mautam' was predicted to occur during the period 2007-2009 and along with the phenomenon an upsurge of rodent population followed by famine also anticipated.

In the context of Northeast India, a biodiversity 'hotspot', no data has been recorded on the spectrum of helminth species infecting rodents. Of the nine genera recorded as rodent pests of agriculture in India, the rodent species recorded from the Northeast region belong to the genera *Funambulas, Rattus, Bandicota*, *Cannomys,* *Mus, Vandeleuria* and *Callosciurus*, with *Bandicota bengalensis* being the most predominant of the prevalent fifteen species.

In view of the seriousness of this problem various government agencies have taken several measures and initiated programme to control rodents. Several studies have been carried out using helminth parasites as a tool for biological control of rodent pests, but none of them have proved to be effective, although some of them have caused certain degree of pathogenesis in their host. Yamaguti (1971) reported about 58 species of trematodes represented by twenty families as parasitizing rodents worldwide. While the cestodes infecting rodents are known to belong to 5 different families and include more than 25 different species, the nematode spectrum is represented by more than 40 species under 15 families. Among the acanthocephalans, only 1 species belonging to the family Moniliformidae has been recorded so far from rodent hosts. In India, sporadic studies on similar lines have been done in different parts of the country. The trematode spectrum in rodents hosts is represented by 8 species, the cestode component comprises 11 species, and of the nematodes, so far 17 species have been reported. However, the use of the nematode, *Capillaria hepatica* as a potential biological agent has been emphasized.

So, keeping in view the importance of rodents as major pests of agriculture and plantations, it was felt desirable that a thorough exploration of the helminths harboured by rodents from various parts of Mizoram State that abounds in bamboo plantations and includes wide range of climatic and altitudinal zones be made.

The proposed study, therefore, was taken up with the aim to explore and typify the helminth parasites diversity in rodent population in Mizoram, the regions of bamboo plantation in particular; to study the distribution pattern and species composition of the parasite groups prevalent and represented in the rodent hosts; to pursue morphological, ultrastructural and/or molecular characterization of the parasites species; and to find out if any of the helminth species emerge(s) as potential zoonosis and/or a tool for biological control of rodents.

Chapter – 1

Status and prevalence of Helminth parasite in rodents

INTRODUCTION

Rodents are one of the most successful, abundant and destructive pests inflicting incalculable losses to standing crops, harvested crops in threshing floors, stored food grains and other commodities. Rodents also harbour a number of helminth parasites, which can be transmitted to human beings and other vertebrates; wild rodents have greater ability than other animals to harbor many zoonotic agents and in combination with their broad spectrum and their neighbourhood to domestic animals and humans, they also serve as a reservoir host and aid in dissemination of these worms, thus causing zoonoses. The helminths of public health importance and harboured by rodents include species of *Trichinella, Angiostrongylus* and *Capillaria* (nematodes); *Hymenolepis, Rallietina* and *Echinococcus* (cestodes); and *Schistosoma, Paragonimus* and *Echinostoma* (trematodes) (Khalil, 1986).

The rodent outbreak has been reported from various parts of the world (Pal, 1993). Two types of 'ratadas', the rainfall and bamboo-flowering-associated rodent eruptions or outbreaks, have been prevalent in South America since the Spanish conquest in the 16^{th} century, though the first record of ratadas, associated with bamboo flowering and subsequent mast seeding at cycle of 30 years, appeared in literature since the late 1800s (Jaksic and Lima, 2003). Outbreaks of '*Nuu khii'* (= rats of the bamboo flower), as reported from the uplands of Laos (Singleton and Petch, 1994 ; Schiller et al., 1999), are believed to be triggered by episodic and synchronized flowering and seeding of certain bamboo species is also reported from Japan (Numata, 1970). The hilly state of Mizoram, which lies in the easternmost corner of India, covers an area of 21081 km^2; of the total geographical area 31% is covered by forests, which is assessed at 6,446 km^2. About 29.03% of the total forest area of Mizoram is covered by bamboo forests, which consist of more than 20 species, covering 9 genera of bamboo, of which *Melocanna baccifera* contributes about 95% of the growing stock of bamboo that has been flowering since the year 2003. The gregarious flowering of bamboo followed by famine has been reported to occur periodically in the following sequences- in the year 1815, 1863, 1911 and 1959. The rodent outbreaks at the time of flowering of a bamboo species, *Melocanna baccifera,* caused tremendous destruction of food crops resulting in famine (Nag, 1999). In this bamboo species the flowering, locally known as '**Mautam**', occurs periodically after every 48±1 years and the phenomenon was expected to occur during the period 2007-2009 after 1959 (Singh et al., 1995). According to the folk belief, the rodents feed on the large quantities of highly nutritious seeds of these dying bamboo trees, which, in turn, are believed to trigger a rapid increase in the birth rate of the rodents leading to their population explosion. Following depletion of the bamboo seeds, the huge populations of rats attack fields of paddy and vegetables causing huge losses and even venture into human dwellings and attacking granaries etc. Thus, occurrence of famine in concurrence with the flowering phenomenon is a real happening and not merely a superstitious belief (John and Nadgauda, 2002).

Several studies have been done on the spectrum of parasites of rodents. Yamaguti (1971) reported about 58 species of trematodes represented by 20 families as parasitizing rodents worldwide. Occurence of *Schistosoma* spp in rodent hosts has also been reported from Thailand and France (Bunnag et al., 1980; Combes and Delattre, 1981). While the cestodes infecting rodents are known to belong to 5 different families and include more than 25 different species (Yamaguti, 1959a; Jawdat and Mahmoud, 1980; Wanas et al., 1993a), the nematode spectrum is represented by more than 40 species under 15 families (Yamaguti, 1959b; Tenora et al., 1982; Magalhaes et al., 1983; Wanas et al., 1993b). Among the acanthocephalans, only 1 species belonging to the family Moniliformidae has been recorded so far from rodent hosts (Yamaguti, 1961).

Studies on the endoparasites of commensal rats has also been carried out in several countries such as Malaysia (Singh and Cheong, 1971; Leong et al., 1979; Yap et al., 1977; Krishnasamy et al., 1980; Chooi and Sani, 1985; Ambu et al., 1996; Syed-Arnez et al., 2006; Paramasvaran et al., 2009). In Korea, several workers have reported on the parasite of rats (Nakamura and Kobashi, 1935; Ogura, 1936; Park 1938; Seo et al., 1964, 1968; Yong et al., 1991; Seong et al., 1995). Hasegawa et al. (1994) and Tung et al. (2009) surveyed the endoparasitic infections in *Rattus rattus*, farm rodents and shrews in Taiwan and reported the occurrence of 4 cestodes, 10 nematodes and 1 protozoan, and recorded the occurrence of *Physaloptera* sp. and *Sarcocystis* spp for the first time from the region.

Simmons and Walkey (1971) reported the occurrence of *Capillaria* and *Hymenolepis* in wild rat, which may be hazardous to barrier - maintained laboratory animals. Joseph (1974) reported on the presence of *Hymenolepis diminuta* in gray squirrel from Indiana. Beveridge and Durette-Desset (1992) reported a new species of trichostrongyloid nematode *Odilia bainae* from *Rattus fuscipes*. Gardner and Schmidt (1988) reported on the genus of *Hymenolepis* from Gophers *Geomys* and *Thomomys* spp and worked on the analysis of four species of *Hymenolepis*. Kia et al. (2001) investigated the endoparasites of rodents in Ahvaz, South West Iran and reported 12 different species of helminth parasites. Gomes et al. (2003) studied the nematode spectrum of marsupials and small rodents from the Brazilian Atlantic forest in the state of Rio de Janeiro, Brazil and reported 14 species of nematodes. In Lithuania, Mazeika et al. (2003) provided a new data on the helminth fauna of rodents, and reported the occurrence of 2 trematode and 8 nematode species from the small rodents and 3 trematodes species and larval form of 3 cestodes from musk rat, *Ondatra zibethicus*. Stojcevic et al. (2004) surveyed the rats in the rural regions of Croatia and reported the presence of 7 helminth species. Goncalves et al. (2006) reported the helminth recovered from hystricognath rodent, *Dasyprocta fuliginosa*. Waugh et al. (2006) reported on the zoonotic potential of helminths of *Rattus rattus* and *R. norvegicus* from Jamaica, recording the presence of 6 nematodes, 2 cestodes and an acanthocephalan. Klimpel et al. (2007a) studied the parasitic fauna of bank vole, *Clethrionomys glareolus*, from the urban region of Germany, which included both the ectoparasites and endoparasites - 4 nematode, 3 insect and 3 arachnid species; these authors also studied the parasitic fauna of two abundant sympatric rodent species from Dormagen in Germany and reported 2 digenea, 5 cestode and 7 nematode and 7 ectoparasite species (Klimpel et al., 2007b). Gomez et al. (2008) reported the presence of 3 nematodes and 1 cestode in the Argentine brown rat, *Rattus norvegicus*. Smales (2009) reported on the helminths of *Melomys rufescens* and *Melomys* sp. from Papua New Guinea and described a new genus and 5 new species in the family Heligmonellidae (Nematoda: Trichostrongyloidea). The presence of helminth eggs has been reported in rodents coprolite collected from historical archaelogical site in Chubut Province, Argentina (Sardella et al., 2009).

Significant work has also been done on the parasites of rodents in the neighbouring countries such as Pakistan, Bangladesh and Sri Lanka. Akhtar (1955) reported *Syphacia lahorea* from squirrel in Pakistan and also revised the genus *Aspiculuris* and reported two new species, *A. (A) lahorica* and *A.* (*Paraspiculuris*) *pakistanica*. Cruz and Sanmugasunderam (1971) described 5 new species of cyclophyllidean cestodes from small mammals in Ceylon. Bilqees and Siddiqui (1981) reported *Hymenolepis fusa* and metacestode of *Taenia taeniaeformis* from *Rattus rattus* in Karachi. Huq *et al*. (1985) studied the helminth parasite of *Rattus rattus, Mus musculus* and *Scapanus latimanus* and reported 6 species of helminth parasites in Bangladesh. Bhuiyan et al. (1996) also worked on the helminth parasites of *Rattus rattus* and *Bandicota bengalensis.* Faiyez-ul-Haque et al. (1990) studied the helminth parasite of *Rattus rattus rufescence* and reported 7 species of helminth parasites in Rawalpindi and Islamabad. Bilqees et al. (2001) described a new genus and new species *Pseudorodentolepis lobata* from *Tatera indica* in Karachi. Sumangali et al. (2007) reported on the helminth parasites of 3 rodent species in Kandy District, Sri Lanka. Rafique et al. (2009) reported the presence of 4 cestodes and 3 nematode species from the rodents captured from Faisalabad, Pakistan.

In India, several studies have been carried out on the parasites of rodents from many parts of the country (Balchandra and Ranade, 1975, 1978; Nama and Parihar 1976; Niphadkar, 1980; Singhvi and Johnson, 1980; Johnson et al., 1987; Thakur, 1994; Singla et al., 2008). As per the reports in literature, the trematode spectrum is represented by eight species which belong to the genera *Schistosoma, Orientobilharzia, Brachylaima, Artyfechinostomum, Isthmiophora* and *Haplorchis* (ref. Yamaguti, 1971); cestodes component comprises eleven species under the genera *Mesocestoides, Raillietina, Mathevotaenia, Hymenolepis, Taenia* and *Sudarikovina* and the metacestode *Cysticercus fasciolaris* (ref. Yamaguti,1959a; Raja, 1974 ; Malhotra et al., 1984; Malhotra and Capoor, 1987; Malhotra and Nanda, 1988; Bhattacharya et al., 1998; Jithendra and Somvanshi, 1999; Bhelonde and Ghosh, 2002; Sivakumar et al., 2003). Of the nematode infections, so far seventeen species have been reported belonging to the genera *Trichuris, Capillaria, Syphacia, Subulura, Protospirura, Rictularia, Thelazia, Aspicularis, Angiostrongylus, Heterakis* and *Brienlia* (ref. Yamaguti, 1959b; Saxena and Nama, 1977; Gupta and Dutt, 1981; Sood and Prashad, 1981; Renapurkar et al., 1982; Mandal and Choudhury, 1984; Manna et al., 1985; Tewari, 1982; Gupta and Trivedi, 1988). The distribution of the acanthocephalan *Moniliformis moniliformis* was also recorded (Singhvi and Johnson, 1981; Hemkar and Renapurkar, 1992; Thakur, 1996).

In context of Northeast India, the region is regarded as a biodiversity 'hotspot'. Keeping in view the importance of rodents as major pests of agriculture and plantations, various measures have been taken to control the outbreak of rodents in Mizoram, particularly during the bamboo flowering period. However, strategies of biological control have not been exploited so far. Exhaustive studies on their parasites have never been undertaken and no data have been recorded on the spectrum of helminth species infecting rodents in the region. In view of their underlying threat as serious pests of crop plants and also as reservoir of zoonoses, the present study was undertaken on the parasite spectrum of rodent hosts in the region to assess the worm load in the burgeoning rodent population of the affected region with special references to identifying plausible infectious agents that could serve as a potential tool for biological control.

MATERIALS AND METHODS

Survey of rodent hosts and recovery of parasites

280 rodents, all of which belong to the family Muridae and represented six genera and nine species, namely *Rattus rattus, R. nitidus, R. norvegicus*; *Bandicota bengalensis*; *Berylmys mackenziei, B.bowersi*; *Mus musculus*; *Niviventer fulvescens* and *Cannomys badius,* were collected from 12 different localities in Mizoram. The rodents were collected using snap trap, locally made trap which is also known as 'Vaithang', and glue boards (used for trapping indoors); for live trapping of the rodents different types of live traps including the pit fall trap, Sherman trap or manual trapping using a sack. The various collection sites and their geographical locations are given in Table. 1.1. and Fig. 1.1.

Capture and examination of the animal hosts was done as per the Ethics Committee norms. Different organs such as liver, intestine, lungs, urinary bladder etc were examined for recovery of the helminth parasites. The parasites recovered were duly processed for suitable whole mount preparations following standard procedures. The parasites were fixed and preserved in neutral buffered formalin and 70% alcohol for further morphological studies.

Table 1.1. Collection sites of different rodent hosts in Mizoram

Sl. No.	Collection site	Latitude	Longitude	Altitude
1	Kolasib	24° 13” 25‘N	92° 40” 39’E	2066 ft
2	Bukvannei	24°20” 63’ N	92° 32” 35’ E	184 ft
3.	Bilkhawthlir	24°11” 49’ N	92° 40” 15’ E	1512ft
3	Hlimen	23° 42” 04’ N	92° 43” 01’ E	3433 ft
4	Samtlang	23° 41” 43’ N	92° 43” 07’ E	3173 ft
5	Lungleng	23° 41” 01’ N	92° 42” 55’ E	3415 ft
6	Aizawl	23° 43” 38’ N	92° 44” 11’ E	2389 ft
7	Khawzawl	23° 31” 14’ N	93° 11” 15’ E	3971 ft
8	Darlawn	23° 58” 48’ N	92° 59” 34’ E	955 ft
9	Sawleng	23° 54” 20’ N	92° 59” 20’ E	2139 ft
10	Kepran	23° 56”09’ N	92° 52” 13’ E	525 ft
11	Lunglei	22° 57” 27’ N	92° 48” 54’ E	2914 ft

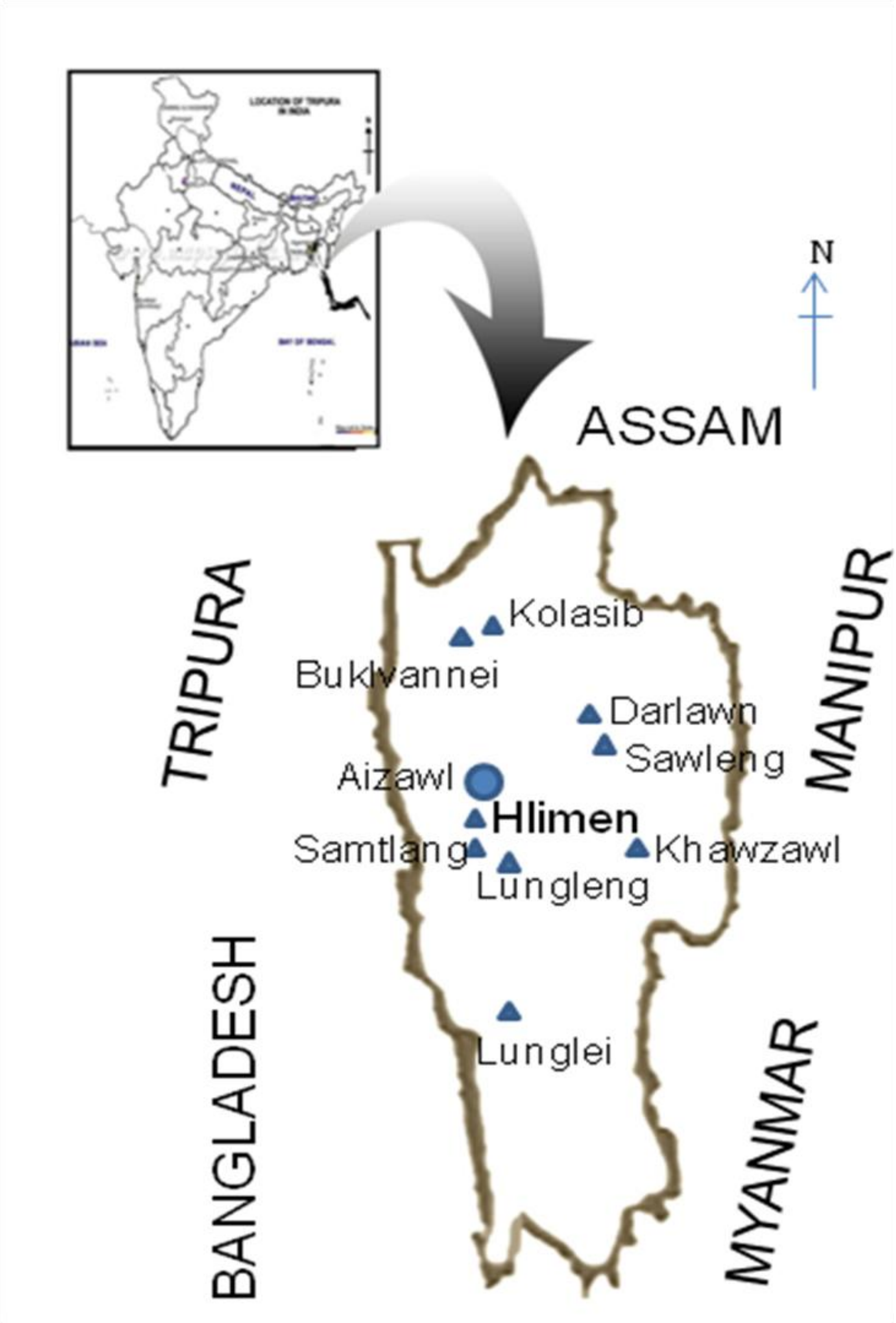


Fig.1.1. Map of Mizoram showing different collection sites of rodent hosts

Light microscopy (LM)

Cestode: Freshly recovered parasites were stretched in warm water and gently flattened between a slide and a cover glass and fixed in 70% ethyl alcohol. The specimens were stained with Borax carmine, and dehydrated through ascending grades of ethyl alcohol, then cleared in Methyl benzoate and mounted in Canada balsam using standard protocols.

Nematode: The worms were stretched in warm 70% alcohol. For permanent mounting, the worms were cleared in ascending grades of glycerine and finally double mounted using Kaiser's glycerine jelly (50 ml water+ 80 g gelatin + 50 ml glycerol + 0.1g phenol); for temporary mounting, the specimens were immersed in lactophenol overnight and temporarily mounted using the same solution on the glass slide.

Acanthocephala: The parasites were allowed to relax in hot saline and narcotized by adding a few drops of 70% alcohol and were further processed following usual procedure of flattening and permanent mounting.

All the prepared permanent slides were observed and studied under Wild M5APO stereo microscope, Leica S6D trinocular stereo zoom microscope, Vision Analyzer, Zeiss Image Analyser or Leitz Ortholux-2 research microscope. Measurements of the specimens were taken using stage and ocular micrometers and/or morphometric software in the image analyser. All measurements are in mm unless otherwise stated. Generic identification of the parasites was done following standard reference works of Yamaguti, 1959a,b,1961,1971; CIH Keys to Nematodes I-X (Anderson, Chabaud and Willmot, 1974-1982; Anderson and Chabaud, 1983). CIH Keys to the Cestode Parasites of Vertebrates, (Khalil et al., 1994).

Scanning Electron Microscopy (SEM)

Live 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 and ethanol-amyl acetate mixture to pure amyl acetate. After their final treatment in dry amyl acetate, the specimens were critical-point dried using liquid carbon dioxide. In lieu of critical-point drying, the specimens after washing in phosphate buffer were dehydrated in ascending grades of acetone, treated with tetramethyl silane [TMS-$(CCH3)_4$Si, boiling point 26.3^{o}C, surface tension 10.3 dynes/cm at 20^{o}C] for 10 minutes and TMS was dried off at 25^o C (Roy et al., 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 35CF (JEOL) and LEO 435 VP SEM at electron-accelerating voltages ranging between 10-20 kV.

Analysis of prevalence data

Data were recorded regarding the prevalence and intensity of helminth infection in rodent hosts over a period of one year; the following parameters were used to analyze the data following Bush *et al*. (1997):

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

Mean intensity (of infection; 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; and

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.

OBSERVATIONS / RESULTS

The parasite spectrum: In the present study the collection of helminth parasites, recovered from the various rodent host species in the study area, comprised a not- so-wide spectrum. The latter included 4 species of cestodes, 9 species of nematodes and only a single acanthocephalan; the trematode component was conspicuously absent.

All the species are reported herein with a brief description, morphometric measurements (in tabular form) and remarks for each of them.

CESTODA: Eucestoda

Order: Cyclophyllidea

Family: Hymenolepididae Ariola, 1899

Genus: *Hymenolepis* Weinland, 1858

Hymenolepis diminuta Rudolphi, 1819

(Fig. 1.2)

The collection comprised 310 specimens of this species recovered from 8 different hosts.

Description (based on measurements of 10 mature specimens): All segments broader than long; rostellum unarmed; suckers unarmed; testes three in number, single poral testis separated by ovary from the two aporal ones; cirrus smooth; ovary multilobed; uterus in form of irregular network; vitelline gland lobed; parts of uterine wall retained in gravid proglottid; genital pores unilateral.

Morphometric measurements of *H. diminuta* are given in Table 1.2.

Host: *Rattus rattus, R. nitidus, R. norvegicus, Berylmys mackenziei, B. bowersi, Bandicota bengalensis, Niviventer fulvescens, Cannomys badius.*

Location: Intestine

Locality: Hlimen, Lungleng, Samtlang, Kolasib (Mizoram); lab maintained

Remarks: *H. diminuta* Rudolphi, 1819 is a cosmopolitan parasite, which has been commonly reported from the intestine of rodent hosts worldwide. In India, *H.*

diminuta has been earlier reported by Balachandra and Ranade (1978) from rats of Poona city. Gupta and Singla (1979) repored and described a single specimen from *Mus terricolor*. This species has also been reported by Singla et al. (2008) among the rodents collected from Punjab.

The present study is the first report of the occurrence of this species from Mizoram, Northeast India. *R. nitidus*, *B. mackenziei*, *B. bowersi, N. fulvescens* and C. *badius* are the new host records for *H. diminuta*.

Table 1.2: Morphometric measurements of *Hymenolepis diminuta*

Characters		**Range**	**Mean**	**±SD**
Total body length		14.3-45 cm	26.66	±9.61
Scolex	Length	0.06-0.19	0.132	±0.052
	Breadth	0.09-0.25	0.17	±0.063
Suckers	Diameter	0.03-0.09	0.063	±0.02
Rostellum	Length	0.02-0.15	0.09	±0.044
	Breadth	0.01-0.04	0.025	±0.012
Neck	Length	1.12-3.45	2.215	±0.981
Immature proglottid	Length	0.08-0.3	0.134	±0.086
	Breadth	0.7-1.2	0.999	±0.124
Mature proglottid	Length	0.16-0.25	0.218	±0.034
	Breadth	0.85-2.05	1.663	±0.334
Gravid proglottid	Length	0.34-0.79	0.477	±0.136
	Breadth	1.04-4.03	2.342	±0.906
Testes	Length	0.09-0.21	0.16	±0.04
	Breadth	0.12-0.209	0.172	±0.035
Cirrus sac	Length	0.09-0.39	0.23	±0.081
	Breadth	0.021-0.10	0.048	±0.023
Ovary	Length	0.07-0.23	0.167	±0.053
	Breadth	0.24-0.74	0.423	±0.168
Vitellaria	Length	0.03-0.11	0.079	±0.029
	Breadth	0.02-0.18	0.124	±0.058
Eggs	Length	0.047-0.074	0.058	±0.008
	Breadth	0.044-0.066	0.051	±0.007

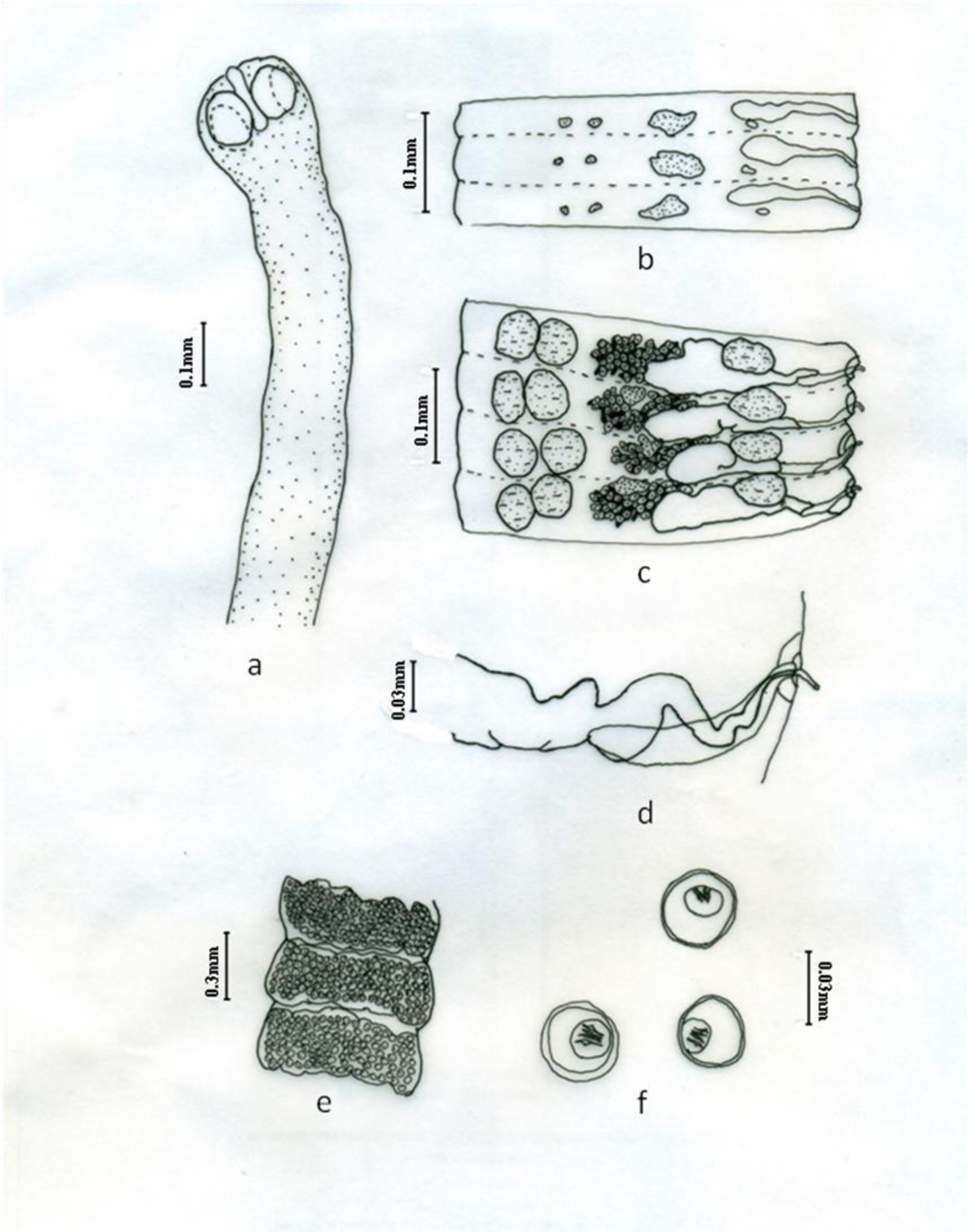


Fig. 1.2 (A). *Hymenolepis diminuta*

a. Scolex with suckers and unarmed rostellum
b. Immature segments
c. Mature segments with reproductive organs
d. Cirrus sac and unilateral genital pore
e. Gravid segments filled with eggs
f. Eggs magnified

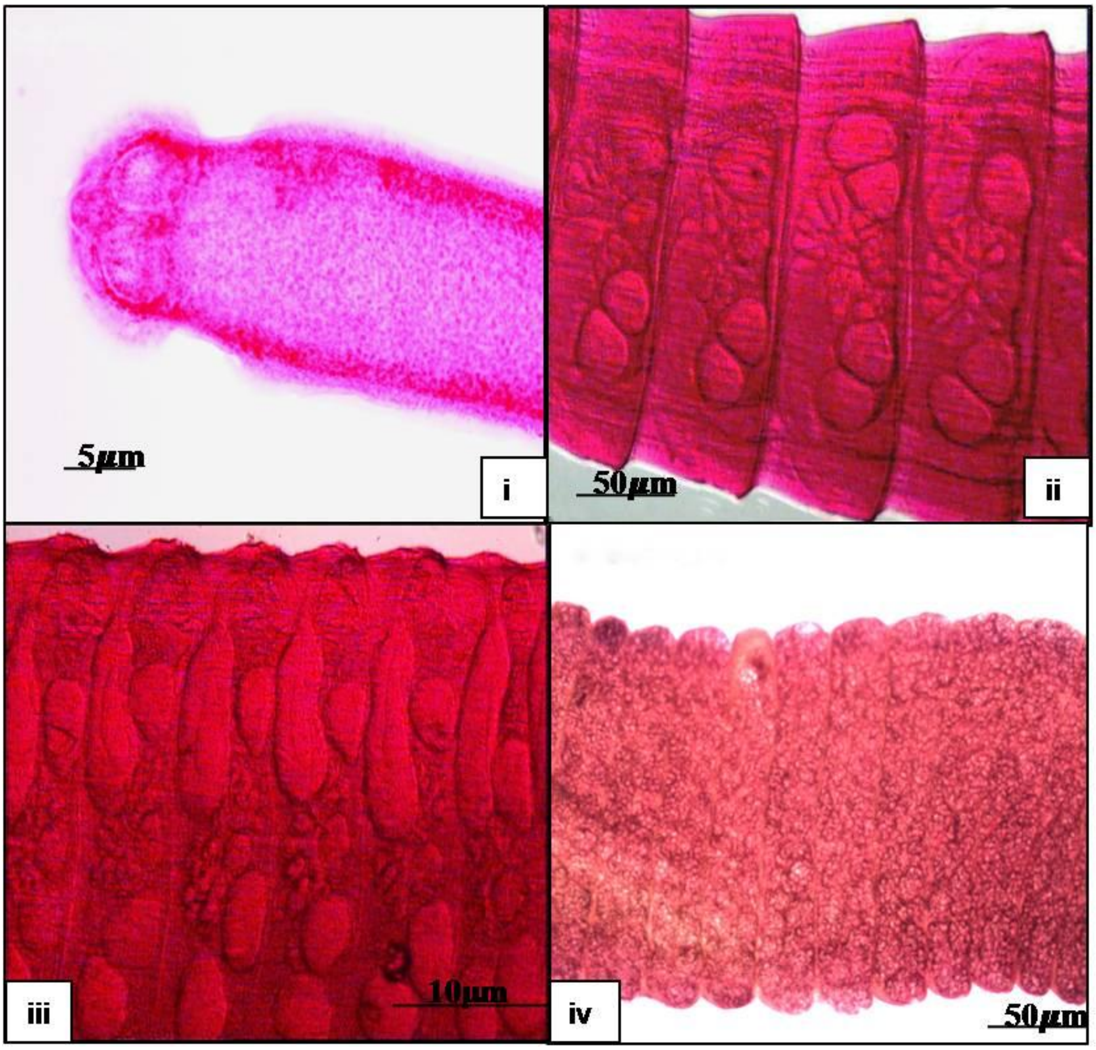


Fig. 1.2 (B). *H. diminuta*- Photomicrographs

i. Scolex with suckers and unarmed rostellum
ii. Mature segments showing ovary and testes
iii. Mature segments
iv. Gravid segments filled with eggs

Genus: *Rodentolepis* Spasskii, 1954

Rodentolepis sp.

(Fig. 1.3)

The collection comprised 28 specimens of this species recovered from 5 different hosts.

Descriptions (based on measurements of 8 specimens): Small worm; strobila with numerous proglottids and progressive maturation; four unarmed suckers; rostellum armed with a crown of wrench-shaped hooks; hooks of nearly identical length; 3 testes- one poral, two anti poral to median vitelline gland; middle testis may overlap vitelline gland; cirrus sac short not reaching middle of proglottid; ovary median; uterus deeply lobate.

Morphometric measurement of *Rodentolepis* sp. are given in Table 1.3.

Host : *R. rattus, R. norvegicus, B. bengalensis, N. fulvescens* and *Mus musculus*

Location: Intestine

Locality: Hlimen, Lungleng, Bilkhawthlir, Darlawn (Mizoram) and also in lab maintained rats

Remarks: The present form belongs to the genus *Rodentolepis*. In India, only one species, *R. bahli,* was reported by Singh (1958) from the musk shrew, *Crocidura caerulea.* However, in the present form that was recovered from different host species, the testes are arranged in a single line, whereas in *R. bahli* they are arranged in a triangle. In morphological features it stands very close to *R. straminea* (Goeze, 1782) Spasskii, 1954, which was reported from *Mus* and *Rattus s*pecies from Russia. However, in the absence of thorough comparison with the type material of *R. straminea*, the species identification of the present form is kept in abeyance.

This is the first report of the genus *Rodentolepis* and *N. fulvescens,* the new host record from Mizoram, Northeast India.

Table 1.3. Morphometric measurements of *Rodentolepis* sp.

Characters		**Range**	**Mean**	**±SD**
Full length of body		2.4-4cm	2.412	±0.301
Scolex	Length	0.09-0.22	0.146	±0.039
	Breadth	0.111-0.24	0.16	±0.049
Suckers	Length	0.035-0.07	0.516	±0.011
	Breadth	0.033-0.065	0.045	±0.01
Rostellum	Length	0.06-0.138	0.144	±0.028
	Breadth	0.022-0.072	0.045	±0.017
No. of rostellar hooks		17-24	19	-
Size of rostellar hooks		0.014-0.016	0.014	±0.0008
Neck	Length	0.36-1.21	0.862	±0.29
Immature proglottid	Length	0.021-0.074	0.043	±0.019
	Breadth	0.096-0.346	0.156	±0.075
Mature proglottid	Length	0.056-0.148	0.094	±0.034
	Breadth	0.131-0.414	0.256	±0.084
Gravid proglottid	Length	0.086-0.261	0.161	±0.067
	Breadth	0.225-0.488	0.327	±0.074
Testes	Length	0.021-0.047	0.032	±0.008
	Breadth	0.026-0.047	0.034	±0.006
Cirrus sac	Length	0.024-0.055	0.039	±0.013
	Breadth	0.008-0.048	0.019	±0.002
Ovary	Length	0.035-0.053	0.043	±0.006
	Breadth	0.062-0.106	0.084	±0.014
Vitellaria	Length	0.02-0.103	0.034	±0.024
	Breadth	0.024-0.11	0.039	±0.025
Eggs	Length	0.016-0.052	0.032	±0.015
	Breadth	0.013-0.047	0.029	±0.015

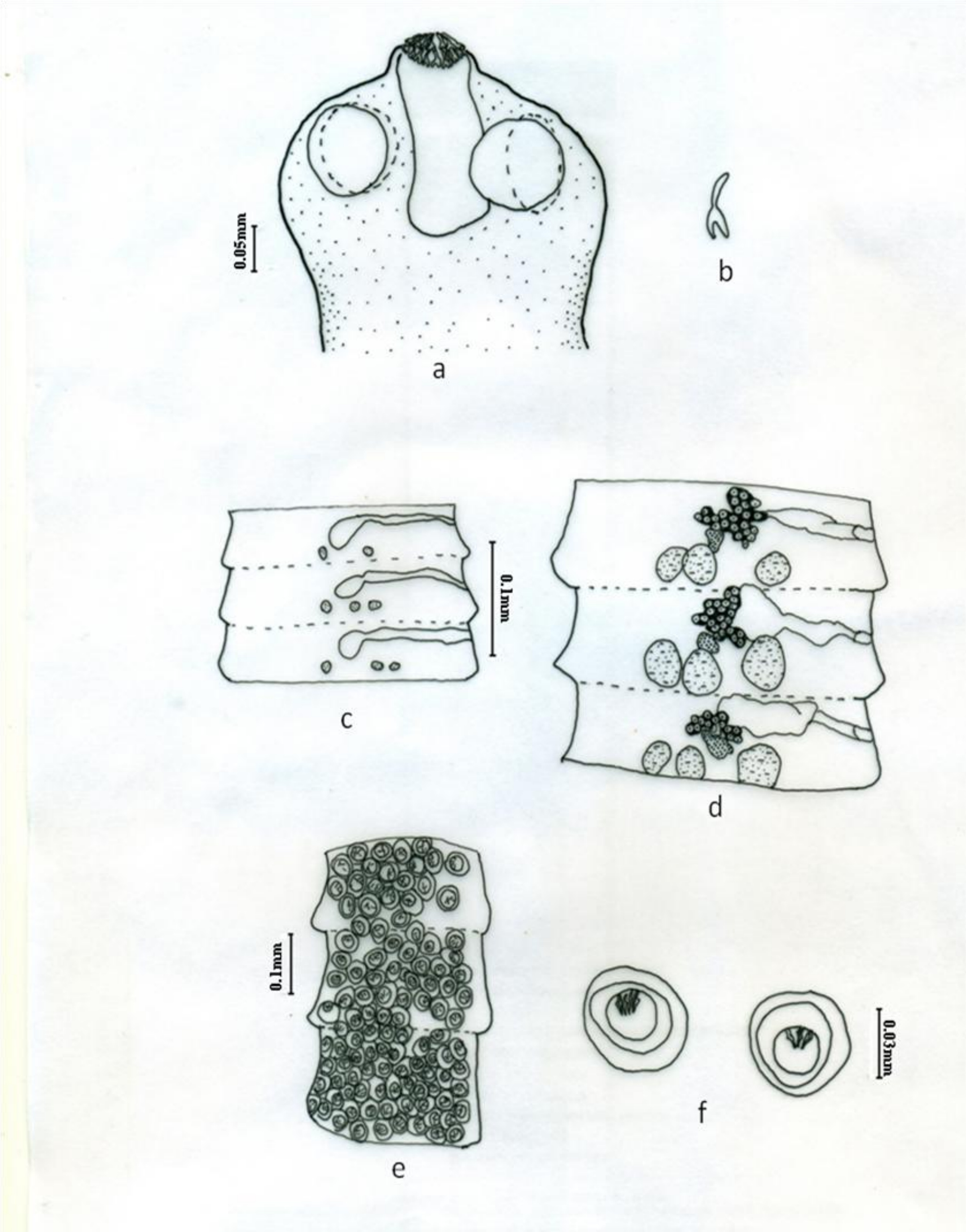


Fig. 1.3 (A). *Rodentolepis* sp.

a. Scolex with suckers and rostellum armed with hooks

b. Hooks magnified

c. Immature segments

d. Mature segments showing ovary, vitellaria and testes

e. Gravid segments filled with eggs

f. Eggs, magnified view

Fig. 1.3 (B). *Rodentolepis* sp- Photomicrographs

i. Scolex with suckers and armed rostellum

ii. Mature segments showing ovary and testes

iii. Gravid segments

Family: Davaineidae Braun, 1900

Subfamily: Davaineinae Braun, 1900

Genus: *Raillietina* Furhmann, 1920

Raillietina celebensis Meggitt, 1927

(Fig. 1.4)

The collection comprised 13 specimens of this species, which were recovered from 4 different hosts**.**

Descriptions (based on measurements of 4 specimen)**:** Scolex with four unarmed suckers; rostellum armed with hammer-shaped hooks arranged in double crown; immediately behind rostellum is spiny collar; reproductive organs in single set per proglottid; cirrus sac small, not reaching or just crossing osmoregulatory canals; testes numerous; ovary median; genital pore irregularly alternate; gravid segment contains numerous egg pouches with egg capsules.

Morphometric measurement of *Raillietina celebensis* are given in Table 1.4.

Host: *R. rattus, R. nitidus, B. bengalensis, N. fulvescens*

Location: Intestine

Locality: Hlimen, Samtlang, Bukvannei (Mizoram)

Remarks: *R. celebensis*, originally reported by Meggit in *Rattus norvegicus* from Burma, is a cosmopolitan parasite. In India this species has been earlier reported by Niphadkar (1977) from rodents of Bombay.

The present study gives the first report of its occurence from Mizoram, Northeast India; *R. nitidus* and *N. fulvescens* constitute new host records for *R. celebensis*.

Table 1.4. Morphometric measurements of *Raillietina celebensis*

Characters		**Range**	**Mean**	**±SD**
Scolex	Length	0.21-0.9	0.366	±0.299
	Breadth	0.309-0.83	0.432	±0.227
No. of rostellar hooks		97-104	101	-
Suckers	Length	0.084-0.122	0.096	±0.014
	Breadth	0.055-0.091	0.0698	±0.013
Neck	Length	1.725-5.29	3.312	±1.466
Immature proglottid	Length	0.115-0.23	0.1814	±0.043
	Breadth	0.764-1.265	0.9818	±0.2
Mature proglottid	Length	0.207-0.465	0.3376	±0.099
	Breadth	1.224-1.615	1.4202	±0.176
Gravid proglottid	Length	0.59-2.139	1.3306	±0.572
	Breadth	1.288-1.748	1.5788	±0.186
Testes	Length	0.048-0.061	0.053	±0.007
	Breadth	0.03-0.05	0.042	±0.0105
No. of testes		23-30	26	-
Cirrus sac	Length	0.069-0.093	0.084	±0.013
	Breadth	0.047-0.062	0.053	±0.007
Ovary	Length	0.102-0.213	0.16175	±0.045
	Breadth	0.225-0.291	0.25375	±0.03
Vitellaria	Length	0.071-0.108	0.0925	±0.015
	Breadth	0.08-0.128	0.0955	±0.022
No. of egg pouch per gravid proglottid		72-90	-	-
Egg pouch size	Length	0.097-0.306	0.2015	±0.147
	Breadth	0.09-0.148	0.119	±0.041
Eggs	Length	0.03-0.114	0.06	±0.046
	Breadth	0.026-0.027	0.026333	±0.0005

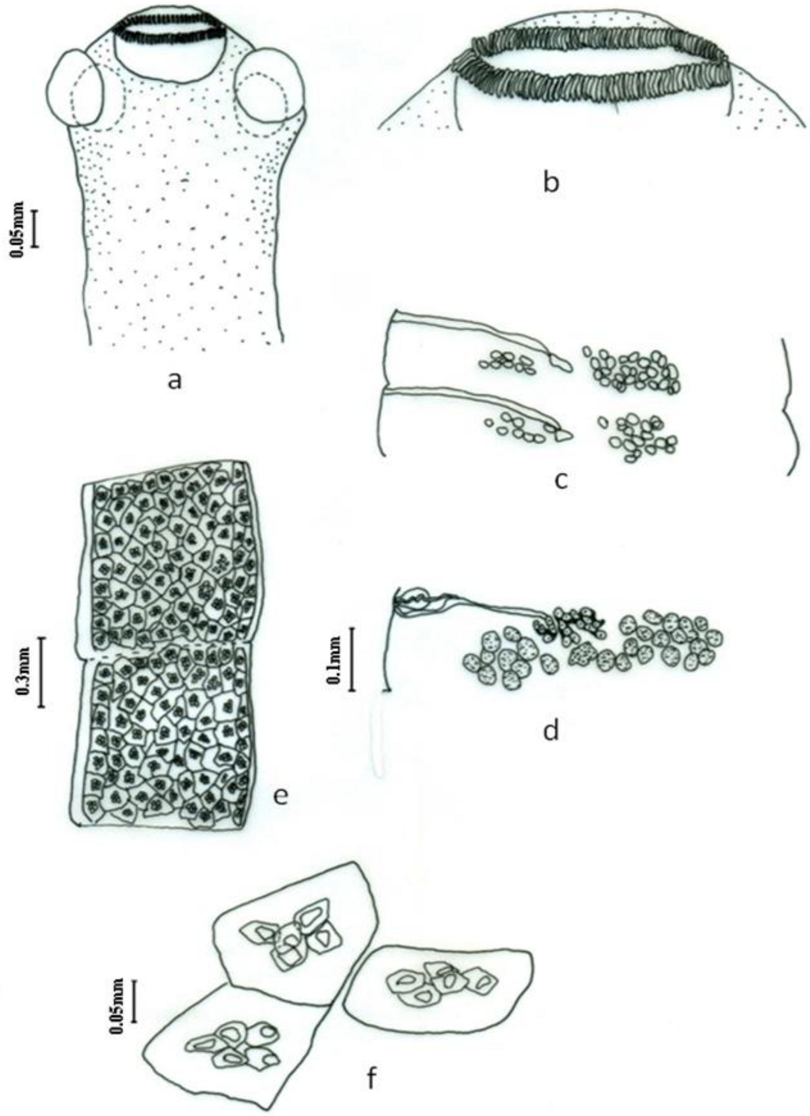


Fig. 1.4 (A). *Raillietina celebensis*

a. Scolex with suckers and rostellum armed with crown of hooks

b. Crown of hooks magnified

c. Immature segments

d. Mature segment showing ovary, vitellaria, cirrus sac and testes

e. Gravid segments filled with eggs

f. Eggs in egg pouches

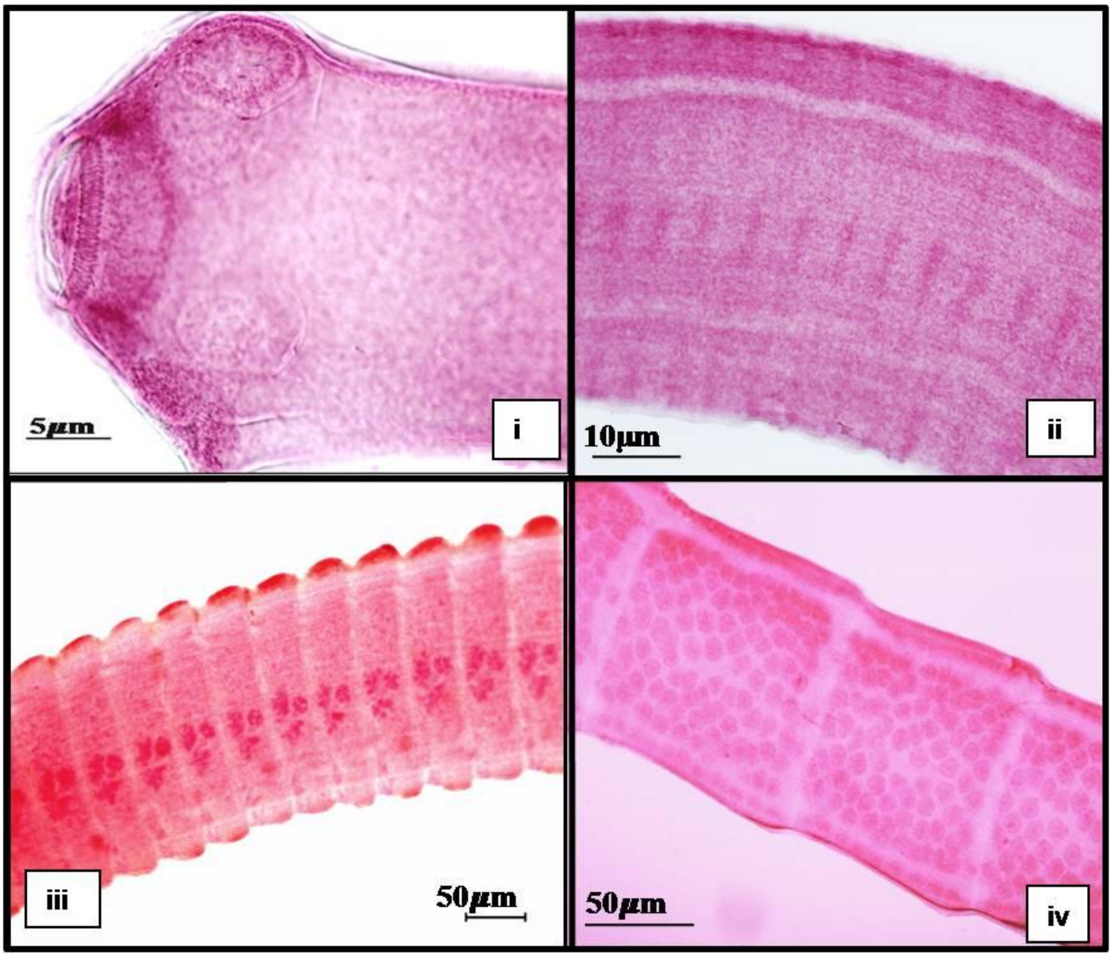


Fig. 1.4(B). *R. celebensis*- Photomicrographs

i. Scolex with crown of hooks

ii. Immature segments

iii. Mature segments

iv. Gravid segments filled with eggs

Order: Taeniidea

Family: Taeniidae Ludwig, 1886

Subfamily: Taeniinae Stiles, 1896

Genus: *Taenia* Linnaeus, 1758

Cysticercus fasciolaris Rudolphi, 1808

(Fig.1.5)

The collection comprised 102 specimens of metacestode which were recovered from 8 different hosts**.**

Descriptions (based on measurement of 12 specimens)**:** Scolex with four prominent lateral suckers; rostellum armed with double row of hooks- inner smaller and outer larger hooks; hooks with typical taenoid features, having long blunt handle with sharp pointed blades; strobila segmented; terminal bladder.

Morphometric measurements of *Cysticercus fasciolaris* are given in Table 1.5.

Host: *R. rattus, R. nitidus, R. norvegicus, B. mackenziei, B. bowersi, B. bengalensis, N. fulvescens, M. musculus*

Location: Liver cyst

Locality: Bilkhawthlir, Lungleng, Kolasib, Hlimen, Samtlang, Sawleng (Mizoram) and also lab maintained

Remarks: *Cysticercus fasciolaris*, the bladder worm larval form of taeniid worms is a cosmopolitan parasite, which has been reported from the liver of rodent hosts worldwide; the adult form *Taenia taeniaeformis* is found in the small intestine of cats and other carnivores. In India it has been reported from different parts of the country, the occurrence of *C. fasciolaris* in palm squirrel and gerbil from Tamil Nadu was reported, Raja, 1974, It was also reported from *R. rattus rufescens* caught in Jodhpur Nama and Parihar, 1976, among the rats in Bombay Niphadkar (1977). Johnson et al. (1987) and Bhattacharya et al. (1998) reported the metacestode from *R. rattus.* Hemkar and Renapurkar (1992) reported its occurrence from *Suncus caeruleus* in Bombay. Jithendran and Somvanshi (1999), Bhelonde and Ghosh (2002) and Sivakumar et al. (2003) have all reported *C. fasciolaris* among laboratory rats.

R. nitidus, B. mackenziei, B. bowersi, and *N. fulvescens* are the new host records for *C. fasciolaris*, which is being reported for the first time from Mizoram, Northeast India.

Table 1.5. Morphometric measurements of *Cysticercus fasciolaris*

Characters		**Range**	**Mean**	**±SD**
Full length of body		0.9-9.6 cm	5.5	±2.569
Scolex	Length	1.05-1.84	1.31	±0.285
	Breadth	1.01-2.09	1.515	±0.334
Suckers	Length	0.28-0.39	0.334	±0.035
	Breadth	0.25-0.35	0.294	±0.032
No. of hooks		34-42	37	-
Size of hooks:	Inner hooks	0.23-0.27	0.248	±0.014
	Outer hooks	0.36-0.43	0.401	±0.019
Proglottid	Length	5.31-93.93	42.99	±26.15
Bladder	Length	1.26-7.01	4.061	±1.709
	Breadth	0.5-7.47	2.933	±2.095

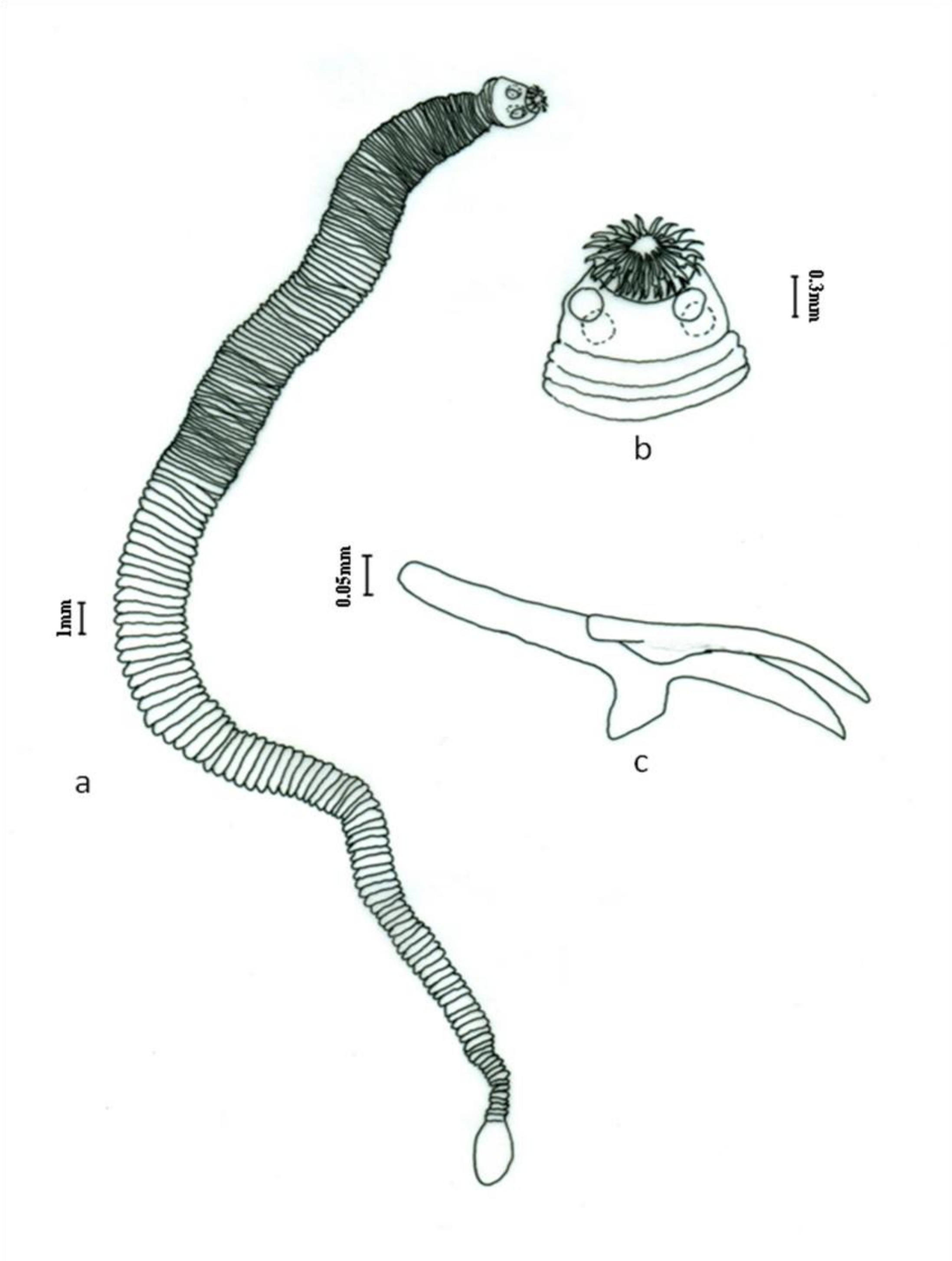


Fig. 1.5 (A). *Cysticercus fasciolaris*

a. Whole worm with armed rostellum and terminal bladder

b. Scolex magnified showing suckers and hooks

c. Hooks magnified

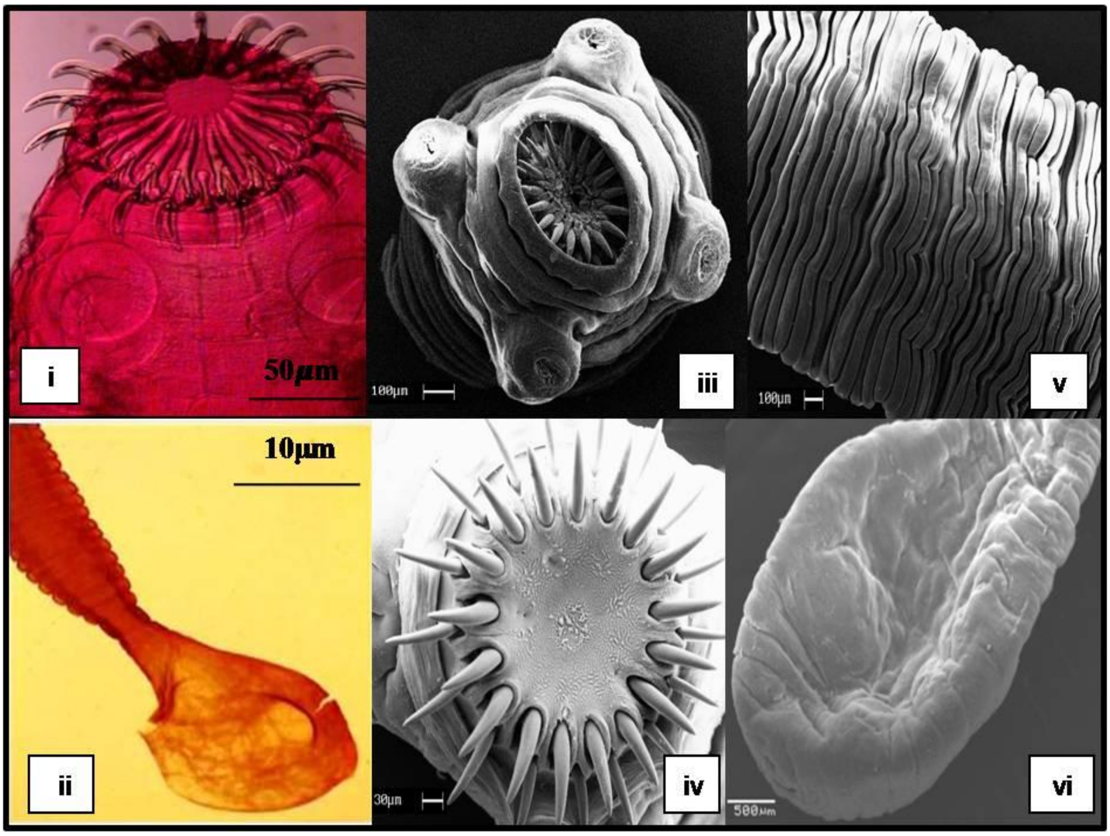


Fig. 1.5 (B). *C. fasciolaris*- Photomicrographs (i, ii- LM) and electron micrographs (iii, iv) SEM

i. iii. iv. Anterior end showing suckers and rostellar hooks

v. Proglottid

ii. vi. Posterior end showing bladder

ACANTHOCEPHALA

Order: Moniliformida

Family: Moniliformidae Van Cleave, 1924

Subfamily: Gigantorynchinae Travassos, 1915

Genus: *Moniliformis* Travassos, 1915

Moniliformis moniliformis (Bremser in Rud., 1819) Travassos, 1915

(Fig. 1.6)

The collection comprised 15 specimens (all female) of this species recovered from a single host**.**

Descriptions (based on measurements of 4 (female) specimens)**:** Body long; worms appear segmented due to bead-like pseudosegmentation of tegument; protrusible proboscis cylindrical and armed with small hooks arranged in spiral rows; hook with backwardly directed root; proboscis receptacle comparatively short, double walled with ganglion near or at its base, outer wall consisting of spiral muscle; lemnisci filiform, long; Eggs elliptical comparatively large with concentric membrane.

Morphometric measurement of *Moniliformis moniliformis* are given in Table 1.6.

Host: *Rattus nitidus*

Location: Intestine

Locality: Hlimen

Remarks: Among the Acanthocephala, only one genus *Moniliformis* belonging to the family Moniliformidae has been reported from the rodents so far. In morphological and morphometric comparisons the present form is identifiable as *Moniliformis moniliformis,* which has a cosmopolitan distribution. In India, *M. moniliformis* has been reported by various workers such as Raja (1974) from rodents of Tamil Nadu, Balachandra and Ranade (1978) from the rats of Poona city, Singhvi and Johnson (1981) and Johnson et al. (1987) in *R. rattus* from Jodhpur, Thakur (1996) in *R. rattus* from Bihar, Hemkar and Renapurkar (1992) from commensal rats and shrews of Bombay, and also by Singla et al. (2008) from rodents in Punjab.

The present study is the first report of the occurrence of *M. moniliformis* in Mizoram, Northeast, India being a new locality record and *R. nitidus*, a new host record for it.

Table 1.6. Morphometric measurements of *Moniliformis moniliformis* (female)

Characters		**Range**	**Mean**	**±SD**
Full length of body		6.6-17.9cm	10.9	±5.123
Maximum width		1.265-2.415	0.532	±0.532
Collar	Length	0.06-0.105	0.020	±0.020
	Breadth	0.15-0.195	0.025	±0.025
Proboscis	Length	0.378-1.026	0.299	±0.299
	Breadth	0.162-0.189	0.013	±0.013
Lemnisci length:	Right lemniscus	4.149-4.899	3.602	±0.340
	Left lemniscus	3.915-4.508	3.441	±0.252
Eggs	Length	0.066-0.07	0.002	±0.002
	Breadth	0.028-0.031	0.001	±0.001

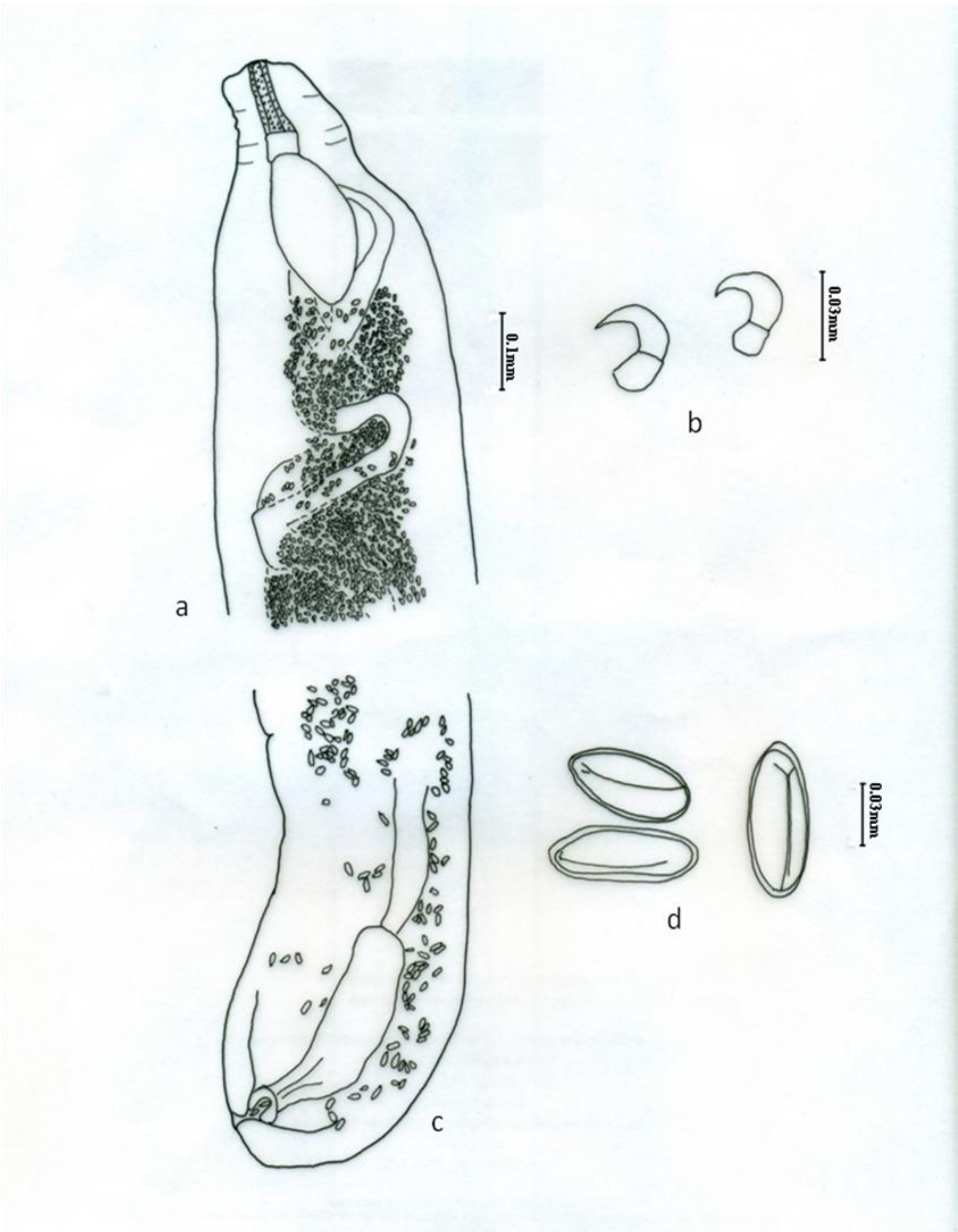


Fig. 1.6 (A). *Moniliformis moniliformis*

a. Anterior end with proboscis and lemnisci

b. Hooks magnified

c. Posterior end showing anal opening

d. Eggs magnified

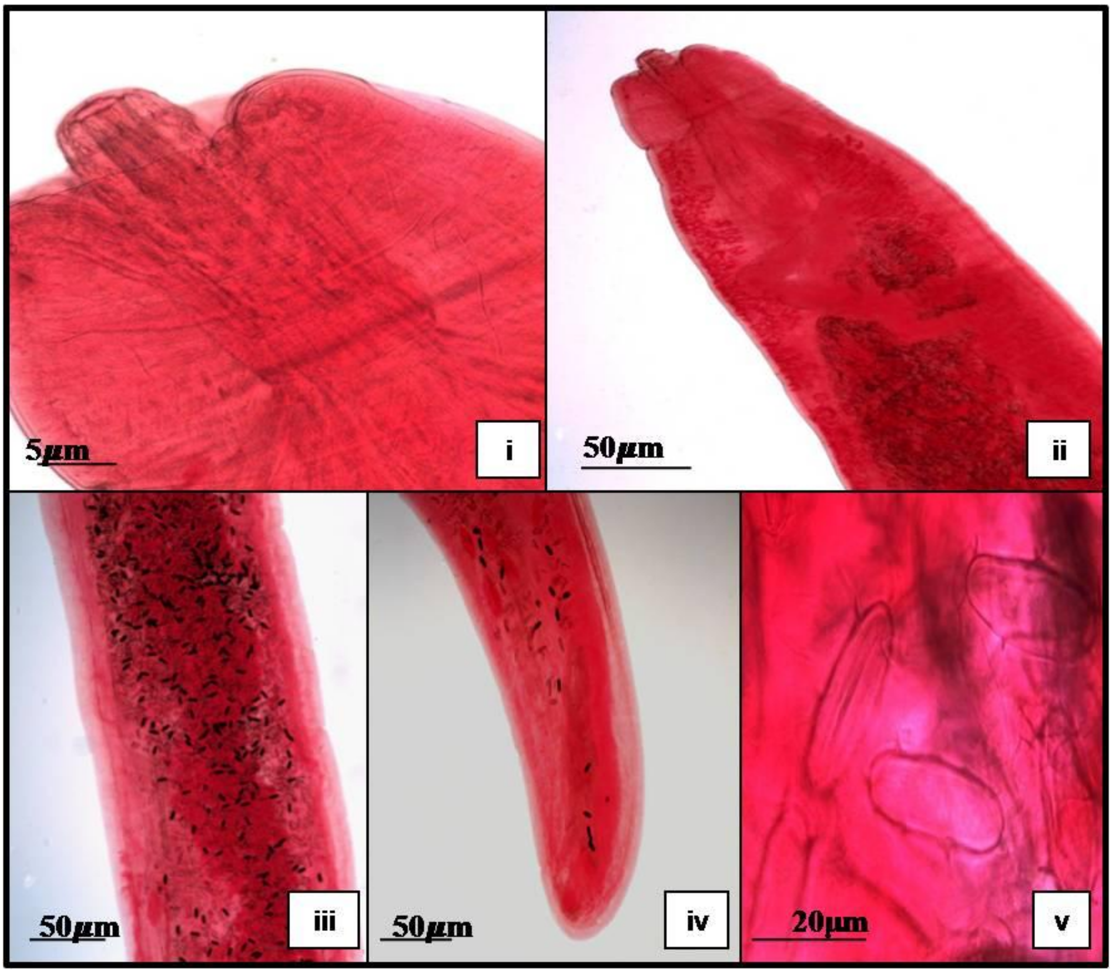


Fig. 1.6(B). *M. moniliformis*- Photomicrographs

i. Anterior region showing proboscis

ii. Anterior region showing lemnisci

iii. Uterus filled with eggs

iv. Posterior region

v. Eggs, magnified view

NEMATODA: Adenophorea

Order: Enoplida

Superfamily: Trichinelloidea

Family: Trichuridae (Ransom, 1911) Railliet, 1915

Subfamily: Trichurinae Ransom, 1911

Genus: *Trichuris* Roederer, 1761

Trichuris muris (Schrank, 1788) Ransom, 1911

(Fig. 1.7)

The collection comprised 11 specimens of this species, which were recovered from 2 different hosts**.**

Descriptions (based on measurements of 3 female and 1 male specimens)**:** Body with slender oesophageal portion and thicker and shorter posterior portion; mouth simple without lips; stichocytes regularly aligned in a single row in oesophageal region; post-oesophageal region of body markedly thickened, with whip - like appearance, posterior portion bluntly rounded in both sexes.

Female: Posterior extremity slightly curved but not spirally coiled; vulva at junction of two body regions or right after oesophagus; anus sub terminal; eggs lemon shaped, with thick brown shell plug at each pole.

Male: Posterior end curled dorsally; single spicule present, well developed; enclosed in prepuce-like sheath with spiny surface; cirrus ususally with spines or tubercles.

Morphometric measurement of *T. muris* are given in Table 1.7.

Host: *Rattus rattus, R. nitidus*

Location: Caecum

Locality: Hlimen, Darlawn, Bilkhawthlir (Mizoram)

Remarks: The genus *Trichuris* shows a cosmopolitan distribution and comprises about 70 species that parasitize a broad spectrum of dosmestic and wild mammals. *T. muris* is a common caecal parasite of rodents. In India, it has been reported from several parts of the country by various workers- Raina and Kaul (1974) from *R. rattus* in Kashmir, Kulkarni and Deshmukh (1984) from Maharashtra, Thakur (1997) from northern Bihar and Hemkar and Renapurkar (1992) in *Suncus caeruleus* from suburbs of Bombay.

The present study gives the first report of *T. muris* from Mizoram, Northeast India and *Rattus nitidus* is the new host record for the species.

Table 1.7. Morphometric measurements of *Trichuris muris*

Female	**Range**	**Mean**	**±SD**
Body length	27.071-30.774	28.794	±1.862
Body width at the level of posterior region of body	0.483-0.506	0.490	±0.013
Oesophagus Length	16.169-17.94	17.165	±0.906
Vulva from anterior end	16.261-18.009	17.248	±0.895
Eggs Length	22-25.75	24.25	±1.984
Breadth	11.75-13	12.583	±0.721
Width at anal opening	0.135-0.153	0.141	±0.01
Male			
Body length	17.342		
Body width	0.46		
Oesophagus Length	8.97		
Spicule Length	0.855		
Breadth	0.036		

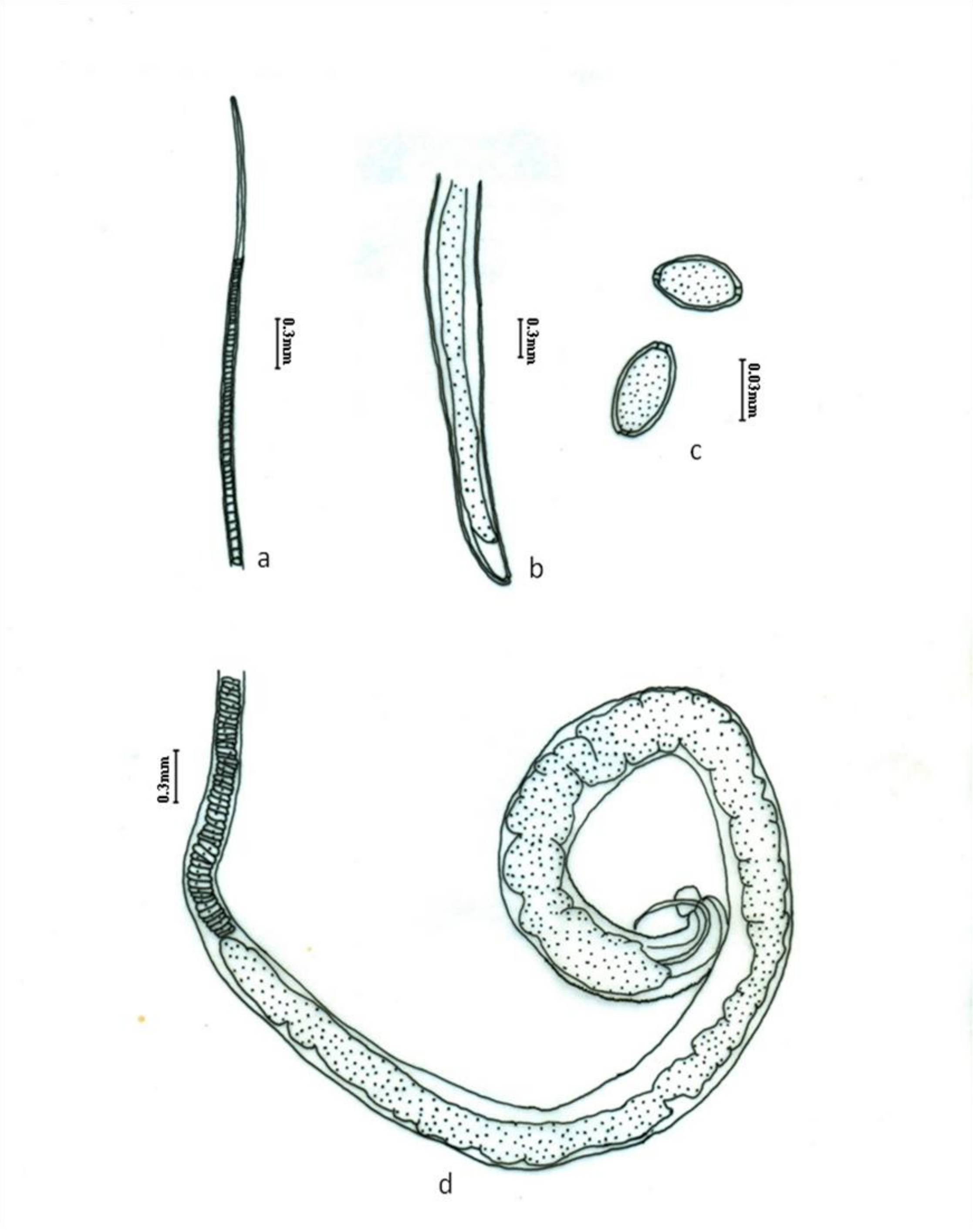


Fig. 1.7 (A). *Trichuris muris*

a. Slender oesophagus

b. Posterior end of female

c. Eggs magnified

d. Male showing oesophageal intestinal junction and single spicule

Fig. 1.7 (A). *T. muris*- Photomicrographs

i. Anterior slender oesophagus giving appearance of whip

ii. Oesophageal intestinal junction (arrow)

iii. Female posterior end

iv. Posterior end of male showing spicule with sheath

Subfamily: Capillarinae Railliet, 1915

Genus: *Capillaria* Zeder, 1800

Capillaria hepatica (Bancroft, 1894) Travassos, 1915

(Fig. 1.8)

Description: Yellowish patches as the worm deposits masses of eggs on the liver surface. Body very slender; Oesophagus with stichocytes; well developed ventral and bacillary bands; sometimes eggs only indication of infection**;** worms disappear after oviposition; eggs with bipolar plugs, outer layer of shell traversed by numerous rod like structure giving radially-striated appearance, measuring 0.046-0.05× 0.024-0.028 in size.

Female: Vulva right behind oesophagus, provided with protrusible, membranous funnel like structure.

Male: Posterior extremity of male blunt, without alae; pair of subventral lobes behind cloacal aperture and papilla at the base of lobe; well developed chitinized spicule, spicule sheath membranous and protrusible without spines.

Host: *R. rattus, R. nitidus, B. mackenziei, B. bowersi, N. fulvescens, M. musculus*

Location: Liver

Locality: Lungleng, Hlimen, Darlawn, Khawzawl (Mizoram)

Remarks: *C. hepatica* is a cosmopolitan parasite, which is commonly found in the liver of rodents. Owing to its body being very slender and tightly entangled in the liver tissue, recovery of the whole worm is mostly difficult. In India it has been reported from several parts of the country by various workers eg. Raja (1974), Niphadkar and Sardeshpande (1978), Naidu and Thakare (1980), Somvanshi et al. (1995), Chahota et al. (1997) and Bhattacharya et al. (1998). Raut et al. (2003) reported this parasite among bandicoot rats, *Bandicota indica* and Patel et al. (2004) and Bhattacharya et al. (2005), among the rats in Pondicherry.

The present study gives the first report from Mizoram, Northeast India and *R. nitidus, B. mackenziei, B. bowersi, N. fulvescens* are new hosts reported for *C. hepatica.*

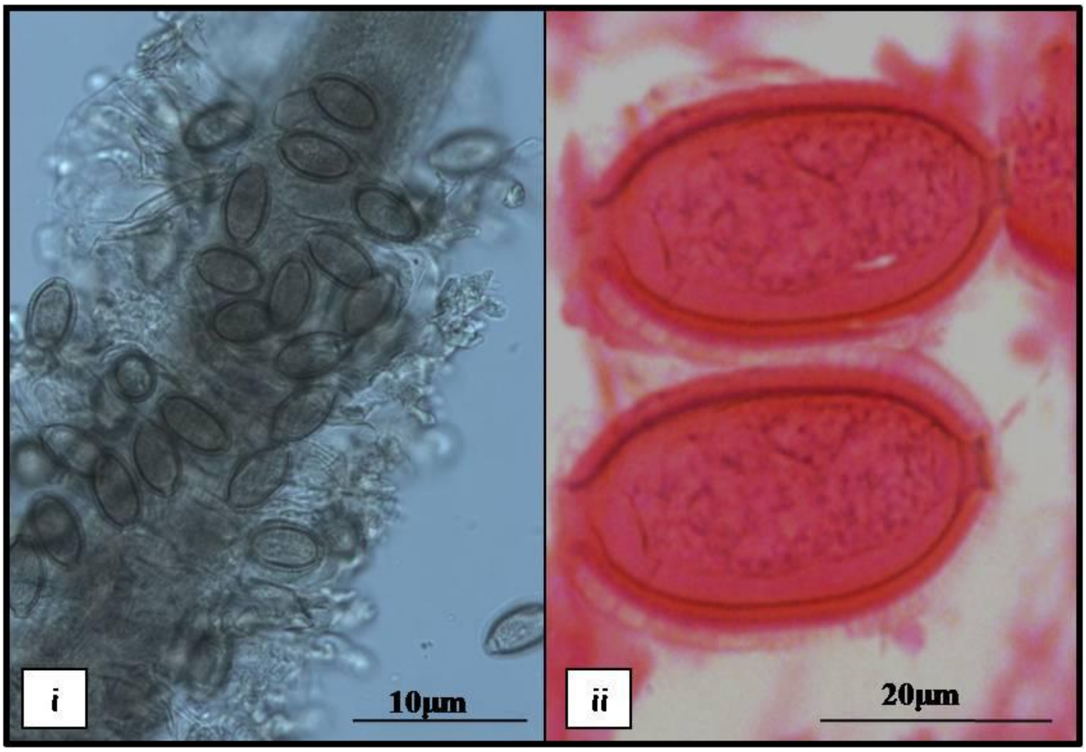


Fig. 1.8. *Capillaria hepatica*- Photomicrographs

i. Clusters of eggs

ii. Eggs, magnified view

Sub family: Trichosomoidinae Hall, 1916

Genus: *Trichosomoides* Railliet, 1895

Trichosomoides crassicauda (Bellingham, 1840) Railliet, 1895

(Fig. 1.9)

The collection comprised 46 specimens of this species, which were recovered from 7 different hosts**.**

Desriptions (based on measurements of 8 specimens)**:** Worm slender; anterior portion of body formed by oesophagus devoid of cell body, remaining portion of oesophagus consisting of cell chain; bacillary band absent; cuticular ornamentation present in form of bosses; anus terminal in both sexes.

Female: Vulva opens behind oesophagus; eggs with thick shell, plugs at each end.

Male: Size much smaller than female, male lodged in uterus of female; spicule and cirrus absent.

Morphometric measurement of *T. crassicauda* are given in Table 1.8.

Host: *R. rattus, R. nitidus, R. norvegicus, B. mackenziei, B. bowersi, B. bengalensis, N. fulvescens*

Location: Urinary bladder

Locality: Bilkhawthlir, Lungleng, Sawleng, Hlimen, Kepran (Mizoram); and also in lab maintained hosts

Remarks: *T. crassicauda* (Bellingham, 1840) inhabiting the urinary bladder of rats shows a cosmopolitan distribution. In India it has been reported from various parts of the country. George and Iyer (1981), Gounalan et al. (1999) and Shingatgeri et al. (2000) have reported from laboratory rats.

The present study provides the first report of its occurence from Mizoram, Northeast India. *R. nitidus, B. mackenziei, B. bowersi* and *N. fulvescens* are new host record for *T. crassicauda*.

Table 1.8. Morphometric measurement of *Trichosomoides crassicauda*

Female	**Range**	**Mean**	**±SD**
Total Body length	8.257-12.604	10.807	±1.88
Body width at the level of mid region of the body	0.108-0.189	0.153	±0.031
Diameter at anterior tip	0.012-0.022	0.015	±0.003
Vulva from anterior end	1.62-2.583	2.094	±0.346
Eggs Length	0.052-0.059	0.056	±0.002
Breadth	0.021-0.038	0.032	±0.006
Male			
Length	1.401-1.905	1.728	±0.224
Breadth	0.021-0.072	0.034	±0.025

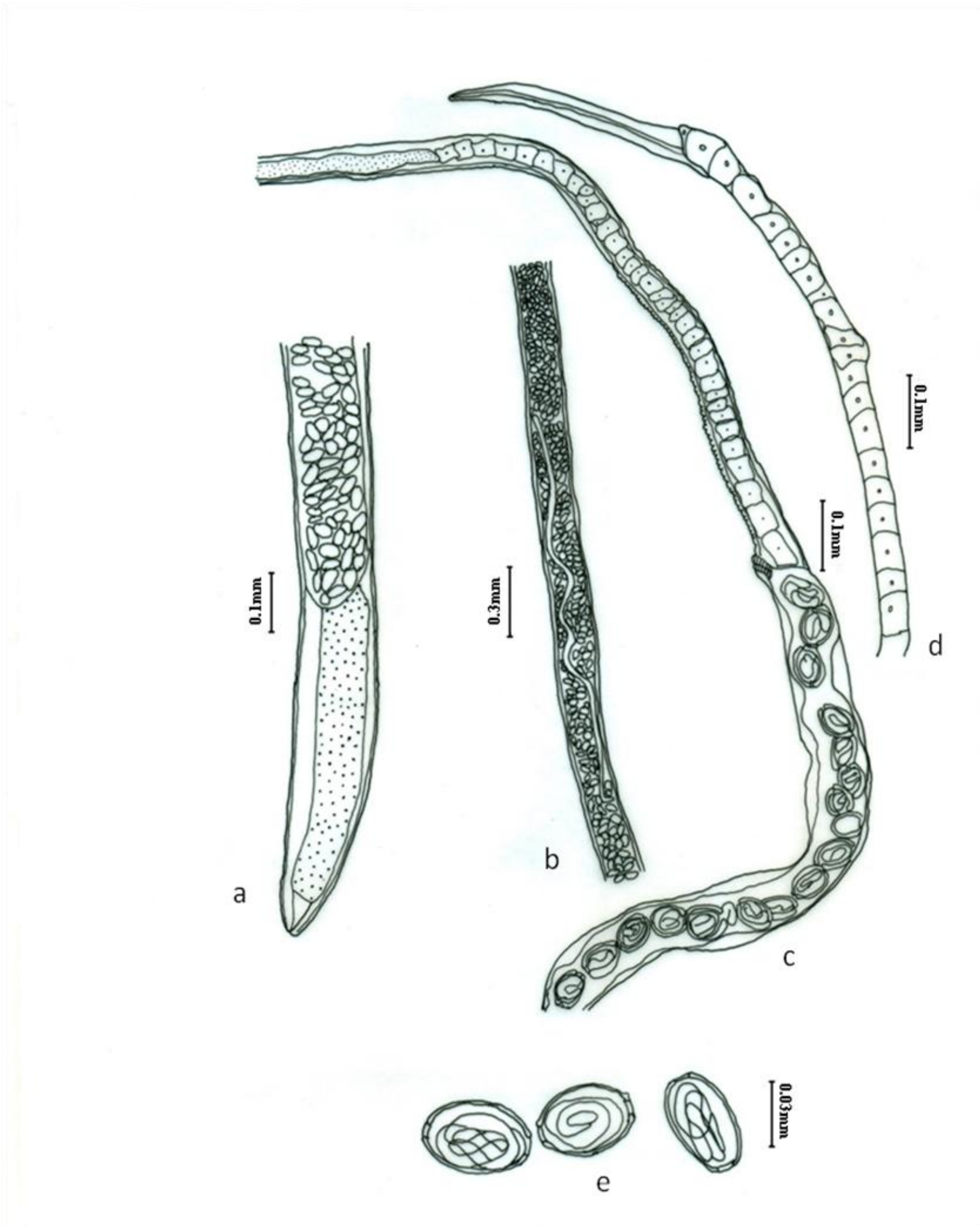


Fig. 1.9 (A). *Trichosomoides crassicauda*

a. Posterior end of female

b. Male in the uterus of female

c. Oesophageal intestinal junction and prominent vulva opening

d. Anterior end

e. Eggs magnified

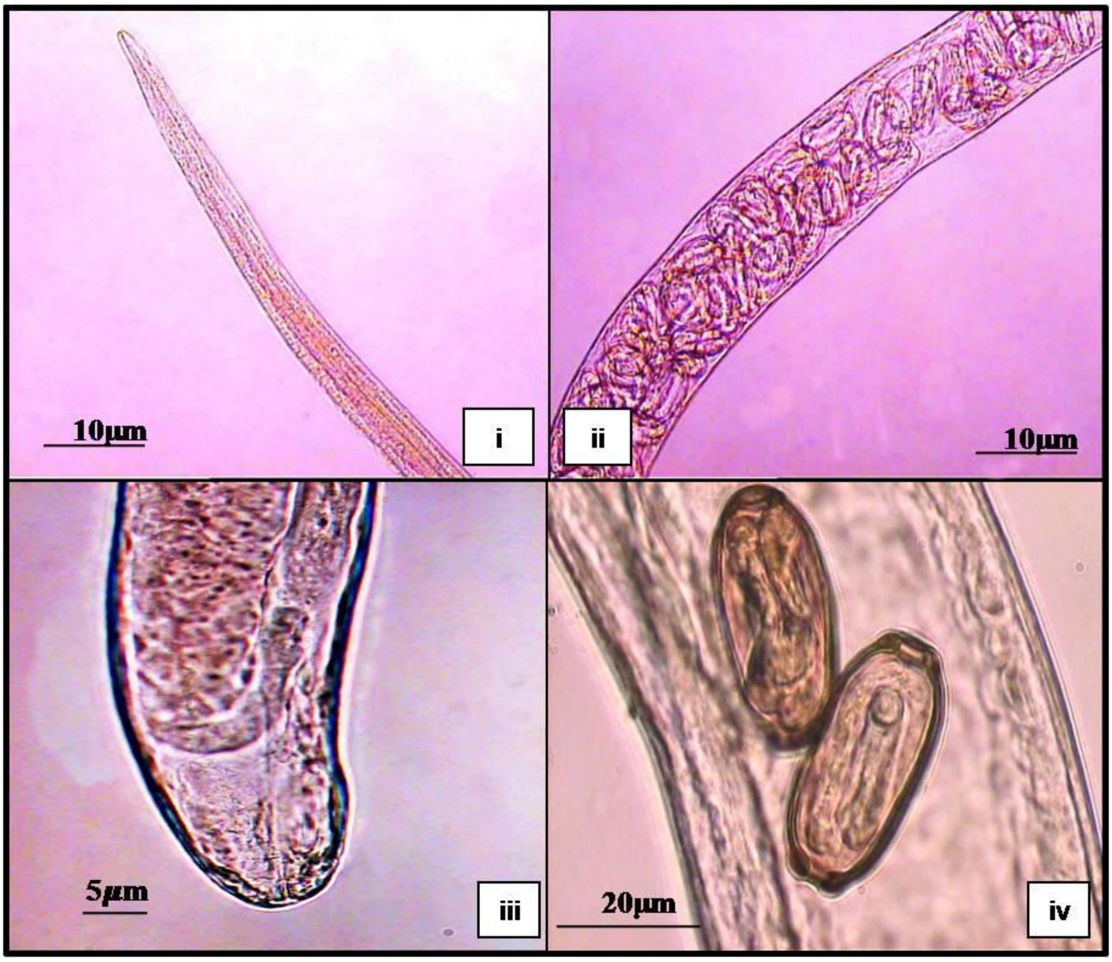


Fig. 1.9 (A). *T. crassicauda*- Photomicrographs

i. Slender anterior end

ii. Uterus filled with eggs

iii. Posterior end of female

iv. Magnified eggs lodged in the uterus of female

NEMATODA: Secernentea

Order: Oxyurida

Superfamily: Heterakoidea

Family: Heterakidae Railliet and Henry, 1912

Sub- family: Heterakinae Railliet and Henry, 1912

Genus: *Heterakis* Dujardin, 1945

Heterakis spumosa Schneider, 1866

(Fig. 1.10)

The collection comprised 135 specimens of this species, which were recovered from 5 different hosts**.**

Descriptions (based on measurements of 15 female and 10 male specimens)**:** Lateral alae extending in male to pre anal sucker, female to tip of tail; alae comparatively wide in cervical region but becoming narrow at about posterior end of oesophagus.

Female: Vulva behind middle of body, prominent lips, each of which bears papillae; eggs with mammiliated shells; tail long, tapering.

Male: Caudal alae wide anteriorly, diminish in width at about middle of tail; ten pairs of caudal papillae, paracloacal group five pairs-three lateral, two subventral; of lateral papillae, the anterior on each side is very large, with stout granular base, the next is smaller with thickened base, while posterior is long but comparatively slender; posterior group situated on more slender portion of tail, consists of three pairs, of which the anterior papilla most slender and middle papilla stoutest; spicules sub equal, flattened, gradually tapering, longitudinally striated.

Morphometric measurements of *Heterakis spumosa* are given in Table 1.9.

Host: *R. rattus, R. nitidus, B. mackenziei, B. bengalensis.*

Location: Intestine, caecum

Locality: Samtlang, Aizawl, Darlawn, Sawleng, Hlimen, Bilkhawthlir (Mizoram)

Remarks: *H. spumosa* is a cosmopolitan parasite, commonly reported from the caecum of rats worldwide. In India this species has been reported from *Rattus norvegicus* from Behrampore, West Bengal. Niphadkar (1977) reported it from *B. bengalensis*, *R. rattus* and *R. norvegicus* from two localities of Bombay and Balachandra and Ranade (1978), from *R. rattus* and *B. indica* from Poona city.

The present study gives the first report of the occurrence of the *H. spumosa* from Mizoram Northeast India and *R. nitidus, B. mackenziei* are its new host records.

Table 1.9. Morphometric measurements of *Heterakis spumosa*

Female	**Range**	**Mean**	**±SD**
Maximum body length	8.87-15.295	11.13	±1.517
Maximum body width at the middle of body	0.082-0.378	0.322	±0.078
Start of lateral alae	0.135-0.207	0.167	±0.021
Oesophagus Length	0.774-1.116	0.909	±0.098
Breadth	0.126-0.198	0.157	±0.016
Nerve ring from anterior end	0.06-0.225	0.085	±0.042
Excretory pore from posterior end	0.285-0.36	0.322	±0.026
Vulva opening	4.66-7.59	5.583	±0.704
Eggs Length	0.044-0.06	0.054	±0.003
Breadth	0.032-0.04	0.037	±0.002
Anal opening from posterior end	0.72-1.09	0.88	±0.099
Tail Length	0.675-1.008	0.82	±0.087
Male			
Maximum body length	7.93-10.02	9.282	±0.747
Maximum breadth	0.27-0.405	0.348	±0.052
Oesophagus Length	0.756-0.936	0.872	±0.06
Breadth	0.135-0.18	0.151	± 0.016
Excretory pore	0.306-0.369	0.334	±0.024
Pre anal sucker Length	0.057 0.096	0.066	±0.011
Breadth	0.078-0.105	0.09	±0.008
Spicules Left Length	0.156-0.29	0.24	±0.043
Breadth	01-.02	0.013	±0.002
Right Length	0.17-0.28	0.0239	±0.035
Breadth	0.012-0.02	0.014	±0.002
Tail Length	0.237-0.315	0.282	±0.028

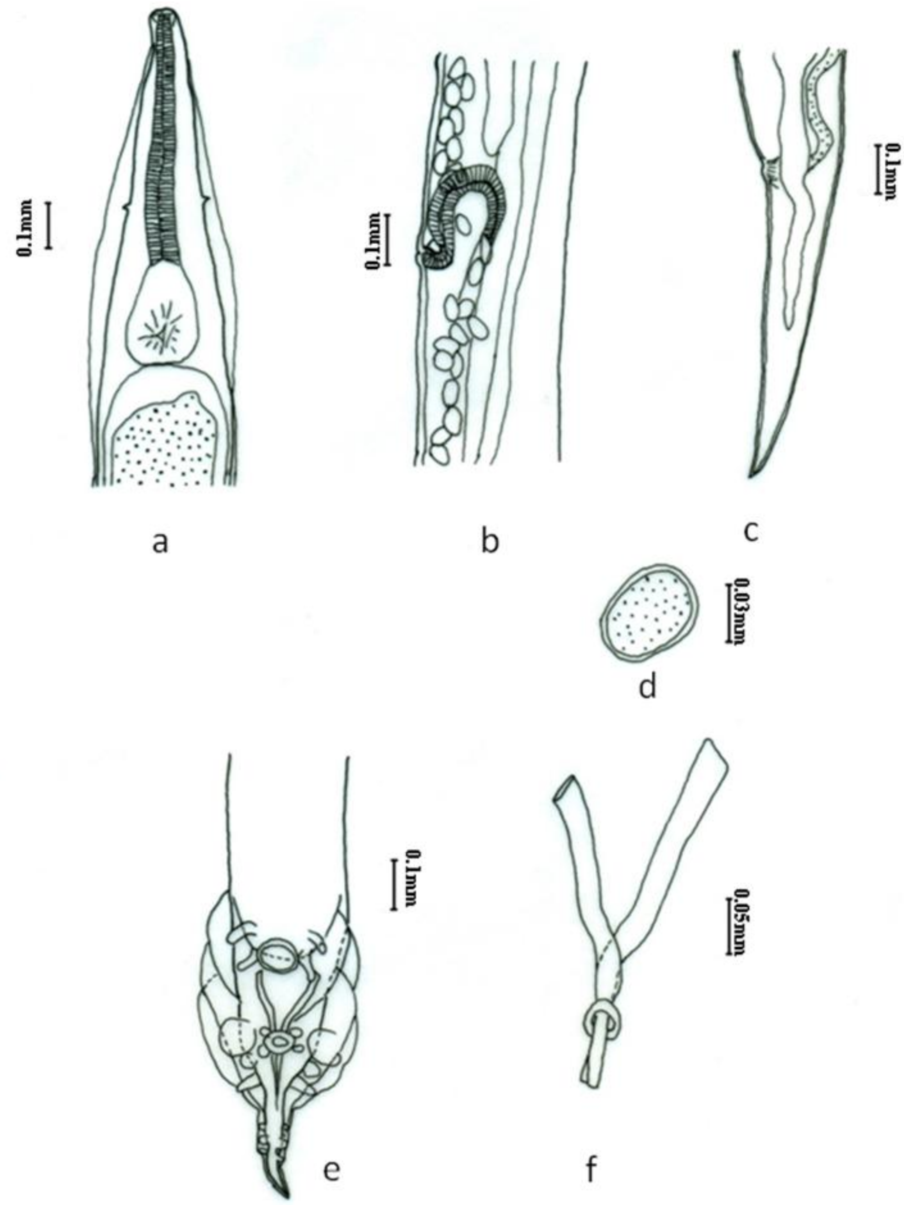


Fig. 1.10 (A). *Heterakis spumosa*

a. Anterior region showing club shaped oesophagus

b. Vulva opening

c. Posterior end of female

d. Eggs magnified

e. Posterior end of male with ten pairs of caudal papillae

f. Spicule magnified

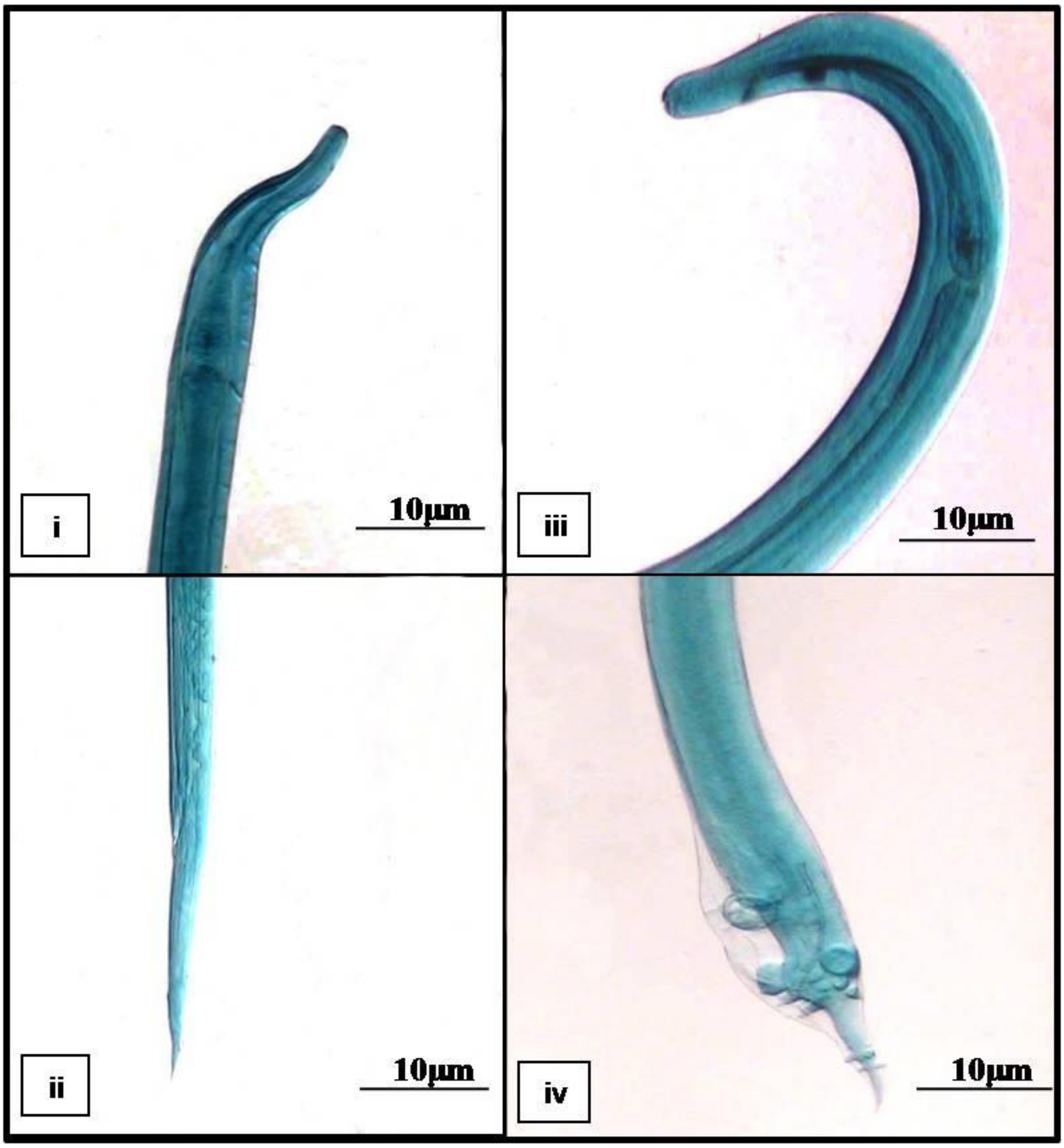


Fig. 1.10 (B). *H. spumosa*- Photomicrographs

i, ii. Anterior and posterior ends of female

iii Anterior end of male

iv Posterior end of male, showing caudal papillae and spicules

Order: Spirurida

Superfamily: Rictularioidea

Family: Rictulariidae Railliet, 1916

Sub Family: Rictulariinae Hall, 1913.

Genus: *Rictularia* Frolich, 1802.

Rictularia sp.

(Fig.1.11)

The collection comprised 15 specimens of this species recovered from 2 different hosts**.**

Descriptions (based on measurements of 10 female and1 male specimens): Buccal cavity well cuticularized, armed at its base with teeth; oral opening hexagonal, inclined dorsally, bordered with small denticles; oesophagus club shaped; body ornamented along its whole length with two longitudinal sub ventral rows of comb-like spines, latter becoming scarier and smaller posteriorly.

Female: Vulva prominent, vulvar lips well developed; uterus packed with eggs, extends posteriorly as far as anus; eggs thick shelled, embryonated, oval to elongate in shape, bluntly pointed with basal spine-like structure.

Male: Caudal region coiled ventrally; spicules unequal; gubernaculum absent; four fan-like processes present at posterior end.

Morphometric measurement of *Rictularia* sp. are given in Table 1.10.

Host: *R. rattus*, *R. nitidus*.

Location: Intestine

Locality: Hlimen, Darlawn, Lungleng, Sawleng (Mizoram)

Remarks: The genus *Rictularia* shows a cosmopolitan distribution. In India, *R. ratti* has been reported from *R. norvegicus* and *R. rattus* (Khera, 1954; Johnson, 1969 a) from Lucknow and Jodhpur. Gupta and Trivedi, (1984) reported *R. bandicoti* from *B. indica* in Udaipur. The present specimen differs from the species reported from India mainly in the number of spines present. Considering only the characters of female, the present form stands close to *Rictularia tani* which was described from *R. norvegicus* in China. However, identification, upto the species level could not be reached due to lack of more male specimens, as only one male was collected in the present study.

The specimen under study was also collected from *R. nitidus* besides *R. rattus,* thus adding a new host for the species.

Table 1.10. Morphometric measurements of *Rictularia* sp.

Female		Range	Mean	±SD
Full length of Body		2.3-3.7cm	2.9	±0.52
Maximum width at middle region of body		0.759-0.92	0.791	±0.049
Buccal capsule	Length	0.06-0.09	0.068	±0.008
	Breadth	0.066-0.099	0.073	±0.009
Nerve ring from anterior end		0.297-0.387	0.358	±0.035
Oesophagus glandular	Length	0.603-0.855	0.674	±0.079
	Breadth	0.072-0.09	0.083	±0.007
muscular	Length	3.06-4.293	3.696	±0.377
	Breadth	0.144-0.18	0.169	±0.014
Vulva from anterior end		3.197-4.738	3.882	±0.497
Eggs	Length	0.035-0.04	0.037	±0.002
	Breadth	0.02-0.038	0.027	±0.004
Starting of spines from anterior end		0.069-0.09	0.077	±0.006
No. of spines		91-95	92.6	±1.264
	Pre Vulvular	41-45	43.7	±1.418
	Post vulvular	46-52	49.4	±1.776
Tail	Length	0.189-0.288	0.253	±0.037
Male				
Full length of Body		5.121		
Maximum width		0.297		
Oesophagus	Length	0.405		
	Breadth	1.458		
Buccal capsule	Diameter	0.04		
Nerve ring from anterior end		0.124		
Starting of spines from anterior end		0.05		
Combs		65		
Spicules	Length	0.06		
	Breadth	0.146		

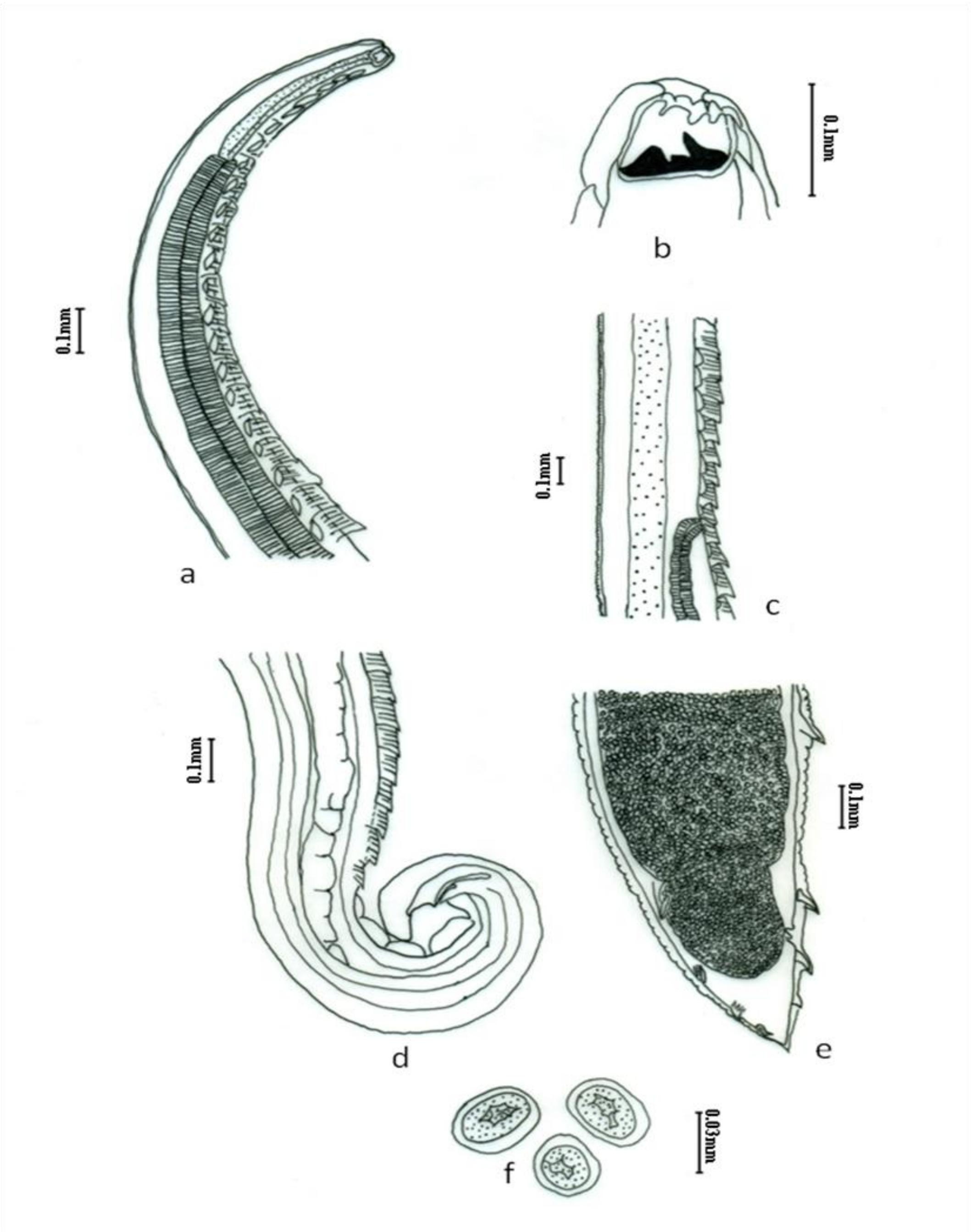


Fig. 1.11(A). *Rictularia* sp.

a. Anterior region showing glandular and muscular oesophagus

b. Anterior end showing teeth

c. Vulva opening

d. Posterior end of male showing unequal spicules

e. Posterior end of female

f. Eggs magnified

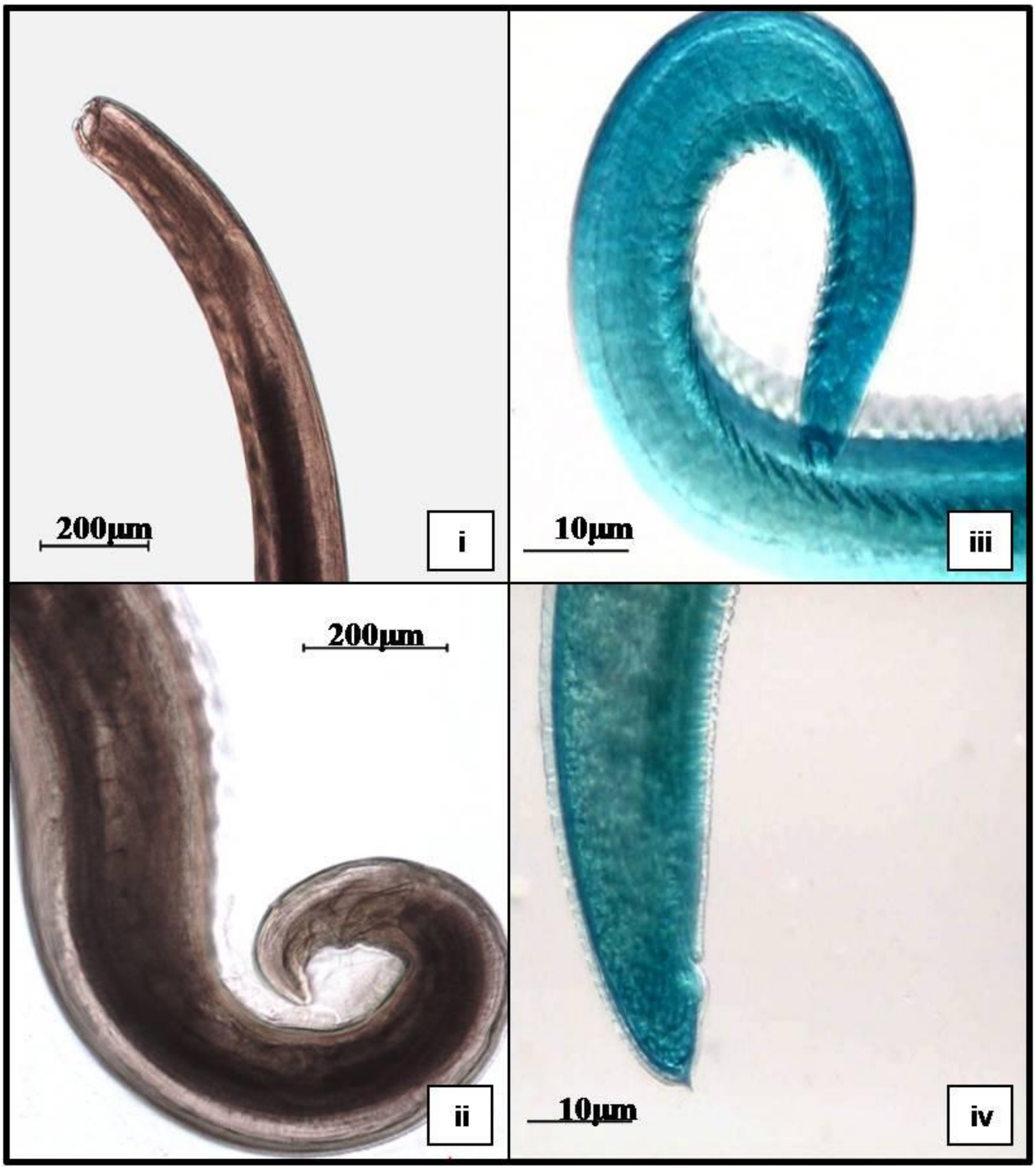


Fig. 1.11(B). *Rictularia* sp- Photomicrographs

i. ii. Anterior and posterior end of male

iii. Anterior end of female

iv. Posterior end showing the uterus filled with eggs and the anal opening

Family: Oxyuridae Cobbold, 1864

Subfamily: Syphaciinae Railliet, 1916

Genus: *Syphacia S*eurat, 1916

Syphacia obvelata (Rudolphi, 1802) Seurat, 1916

(Fig. 1.12)

The collection comprised 127 specimens of this species, which were recovered from 4 different hosts**.**

Descriptions (based on measurements of 10 female specimens**):** Small delicate worm, white in colour; cervical alae present; three distinct lips; buccal capsule absent; oesophagus with short pharynx, prebulbar swelling and distinct posterior bulb; tail of female tapering and pointed; vulva in anterior region of the body post oesophageal; ovijector long, common trunk of uterus relatively long, uterine branches parallel, not reaching up to anus; oviparous; eggs asymmetrical, contain ova not yet fully embryonated in utero.

Morphometric measurements of *Syphacia obvelata* are given in Table 1.11.

Host: *R. rattus, R. nitidus, B. mackenziei, M. Musculus*

Location: Intestine

Locality: Hlimen, Darlawn, Aizawl (Mizoram); also in lab maintained hosts

Remarks: Oxyurid nematodes of the genus *Syphacia* are commonly found in the caecum of rats and mice and are cosmopolitan, pin worms that are commonly used as test organisms in chemotherapeutic studies on enterobiasis.The present form corresponds *Syphacia obvelata* Seurat, 1916. In India, Balachandra and Ranade (1978) reported the same species from rats of Poona city and Nama and Parihar (1980, 1982), from small rodents and the Indian gerbil *Tatera indica* of Jodhpur.

The present study gives the first report of *S. obvelata* from Mizoram, Northeast, India and *R. nitidus* and *B. mackenziei* are new host records for this species.

Table 1.11. Morphometric measurements of *Syphacia obvelata* (Female)

Characters	**Range**	**Mean**	**±SD**
Body length	1.728-2.502	2.098	±0.255
Body width at level of vulva opening	0.234-0.288	0.262	±0.017
Cervical alae Left	0.024- 0.042	0.03	±0.006
Right	0.01-0.04	0.027	±0.009
Oesophagus pharynx	0.126-0.176	0.156	±0.015
	0.036-0.05	0.043	±0.004
Oesophageal bulb	0.072-0.088	0.08	±0.005
	0.074-0.094	0.084	±0.007
Nerve ring from anterior end	0.08- 0.116	0.104	±0.013
Excretory pore	0.279-0.405	0.357	±0.046
Vulva from anterior end	0.333- 0.684	0.537	±0.104
Eggs Length	0.102-0.122	0.114	±0.007
Breadth	0.026-0.04	0.034	±0.005
Anal opening from posterior end	0.17-0.276	0.208	±0.039
Tail Length	0.066-0.189	0.131	±0.061

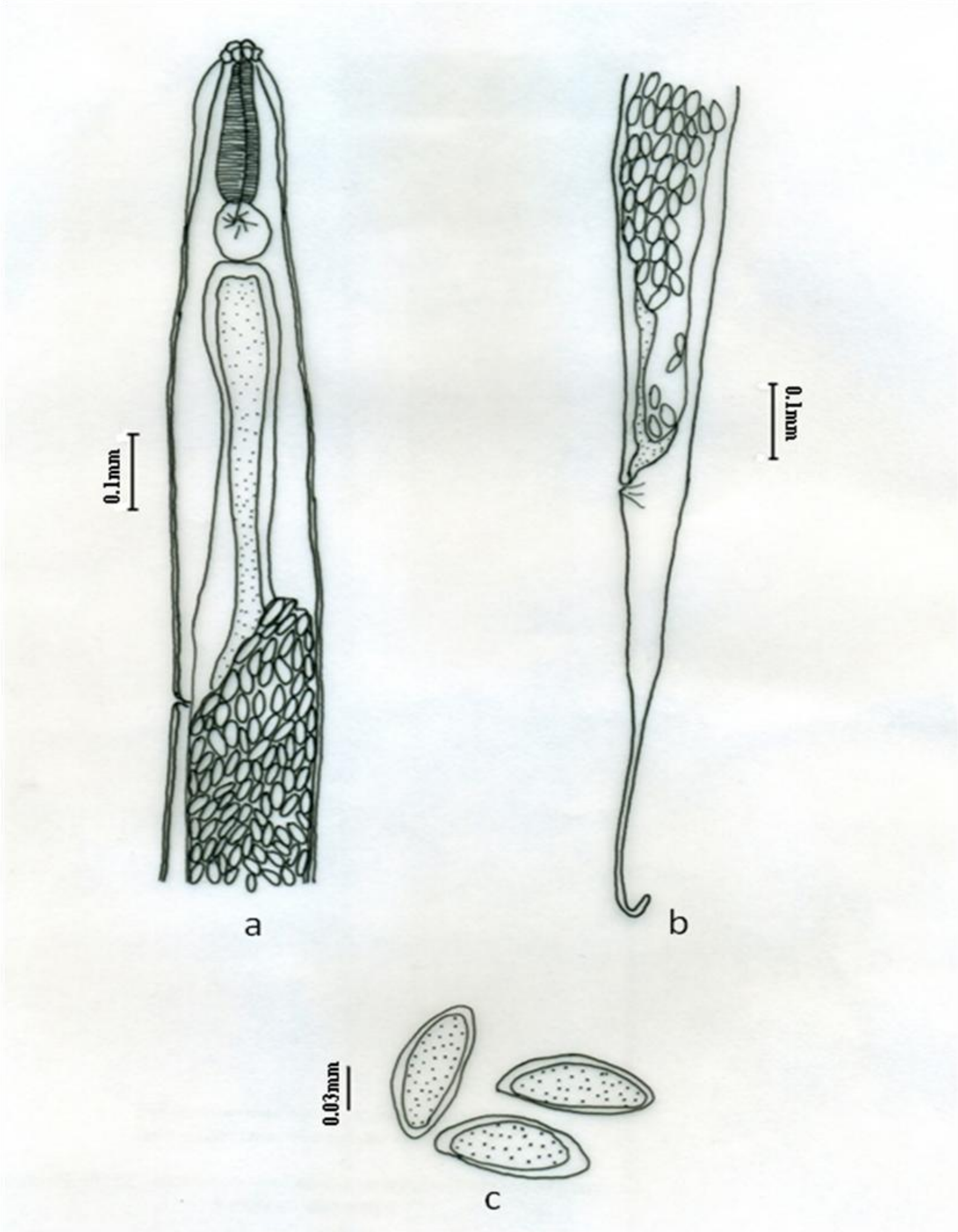


Fig. 1.12(A). *Syphacia obvelata*

a. Anterior region showing prominent lips, oesophageal bulb and vulva Opening

b. posterior region showing the anal opening

c. Eggs magnified

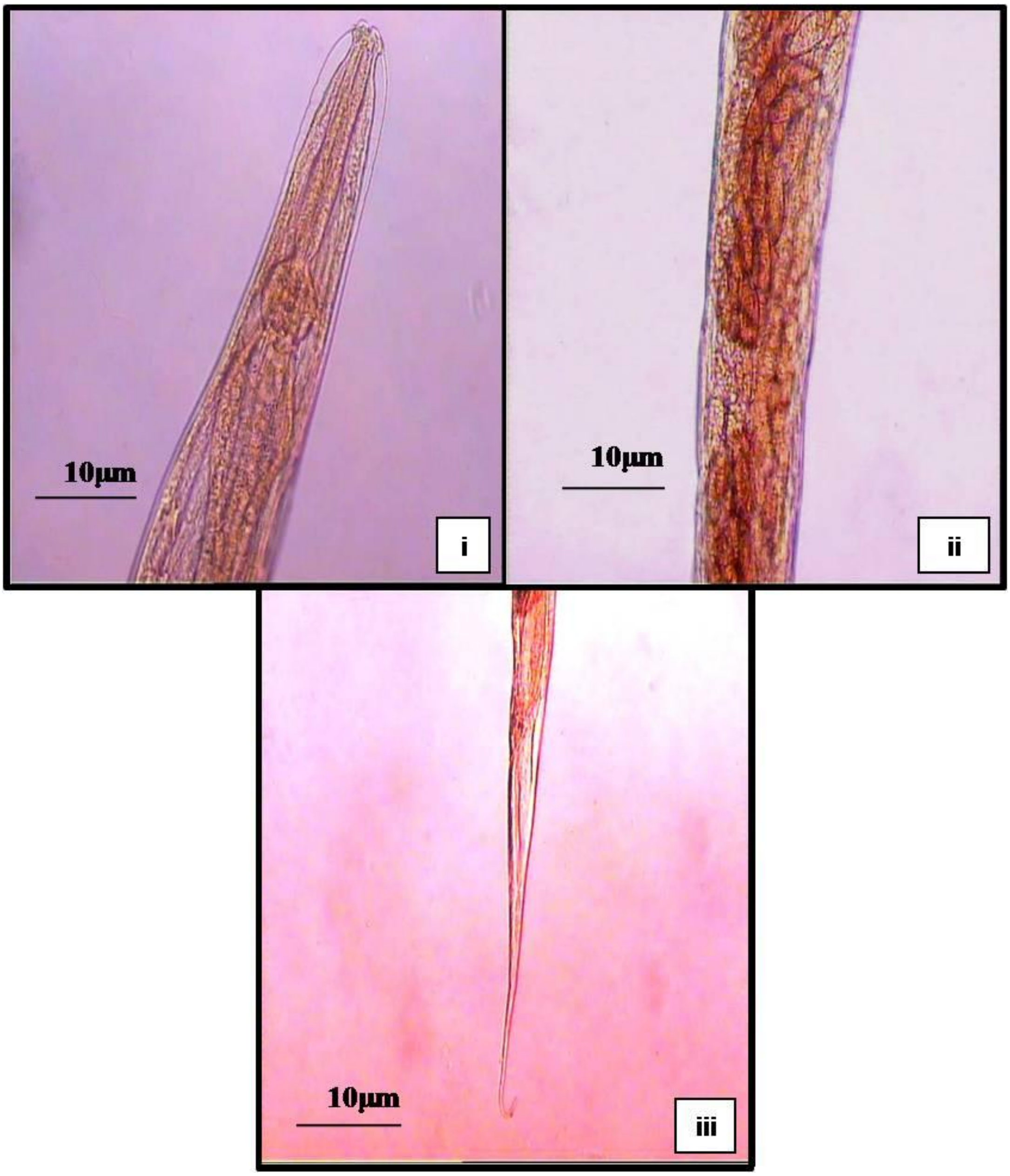


Fig. 1.12(B). *S. obvelata*- Photomicrographs

i. Anterior end

ii. Middle region with eggs

iii. Tapering posterior end of female

Family: Heteroxynematidae Skrjabin and Schikhobalova, 1948

Subfamily: Aspiculurinae Skrjabin and Shikhobalova, 1951

Genus: *Aspiculuris* Schulz, 1924

Aspiculuris (Paraspiculuris) pakistanica Akhtar, 1955

(Fig. 1.13)

The collection comprised 61 specimens of this species which were recovered from 2 different hosts**.**

Description (based on measurements of 10 female and 5 male specimens): Small worms with cuticle striated transversely; mouth surrounded by three lips; head with bulb; cervical papillae small behind nerve ring; cervical alae begin behind head end, terminating in sickle-shaped margin at level of oesophageal bulb and continuing with lateral alae; oesophagus, somewhat club shaped, followed by well developed bulb, which is longer than broad.

Female: Tail conical, tapering; vulva pre equatorial from anterior extremity; vagina runs anteriorly for short distance before taking a posterior course; uterine tube extends beyond the anus; eggs oval with smooth shells containing thick larvae occupying whole length of eggs.

Male: Tail conical, broadly blunted, ventrally curved having caudal processes, caudal alae present, both portion of the tail separately alate; four pairs and two odd caudal papillae present; ventral median rows of laterally compressed cuticular crests in front of the cloaca; spicules and other accessory structures completely absent.

Morphometric measurements of *A. (Paraspiculuris) pakistanica* are given in Table 1.12.

Host: *B. mackenziei*, *M. musculus.*

Location: Intestine

Locality: Darlawn (Mizoram); also in lab maintained hosts

Remarks: One of the most important characters of the genus *Aspiculuris* is the presence of cephalic bulb and the cervical and lateral alae. The present form is placed in the subgenus *Paraspiculuris*, as the cephalic alae are continued with the lateral alae. Several species of *Aspiculuris* have been reported from India. Saxena and Nama, (1977) and Parihar and Nama, (1981) reported *Aspiculuris* (*paraspiculuris*) *pakistanica* from the house rat, *Rattus rattus* and small rodents in Jodhpur. Banerjee and Malhotra, (1988) has reported *Aspiculuris* (*paraspiculuris*) *indicus* from *R. rattus* and *M. musculus* from Garhwal and Johnson (1969 b) reported *A* (*P*) *ratti* from *R. rattus* in Jodhpur. In comparing the morphology and morphometric measurements the present form is identified as *Aspiculuris* (*Paraspiculuris*) *pakistanica,* which was originally described by Akhtar (1955) from rats and mice in Pakistan.

The present study gives the first report of *A.* (*P.*) *pakistanica* from rodents of Mizoram, Northeast India, with *B. mackenziei* as a new host record.

Table 1.12. Morphometric measurement of *Aspiculuris* (*Paraspiculuris*) *pakistanica*

Female		Range	Mean	±SD
Max body length		3.26 - 4.34	3.683	±0.329
Max body width at mid region of body		0.09 - 0.276	0.1991	±0.0519
Diameter at anterior end		0.066 - 0.087	0.078	±0.007
Nerve ring from anterior end		0.066 - 0.09	0.077	±0.01
Oesophagus pharynx	Length	0.15 - 0.207	0.179	±0.019
	Breadth	0.033 - 0.045	0.038	±0.003
bulb	Length	0.09 - 0.129	0.106	±0.012
	Breadth	0.072- 0.09	0.081	±0.006
Vulva from anterior end		1.357-1.65	1.491	±0.079
Eggs	Length	0.068 - 0.084	0.076	±0.004
	Breadth	0.03 - 0.04	0.034	±0.003
Anal opening from posterior end		0.405 - 0.585	0.506	±0.062
Tail	Length	0.405 - 0.55	0.478	±0.054
Male				
Max body length		2.64-3.31	2.954	± 0.265
Max body width at mid region of body		0.117-0.18	0.147	± 0.022
Diameter at Anterior end		0.066-0.078	0.004	± 0.069
Nerve ring from anterior end		0.072-0.099	0.088	± 0.011
Oesophagus pharynx	Length	0.156-0.168	0.161	± 0.004
	Breadth	0.03	0.03	± 0
Oesophageal bulb	Length	0.09-0.105	0.097	± 0.005
	Breadth	0.057-0.063	0.06	± 0.002
Anal opening from posterior end		0.12-0.132	0.124	± 0.005

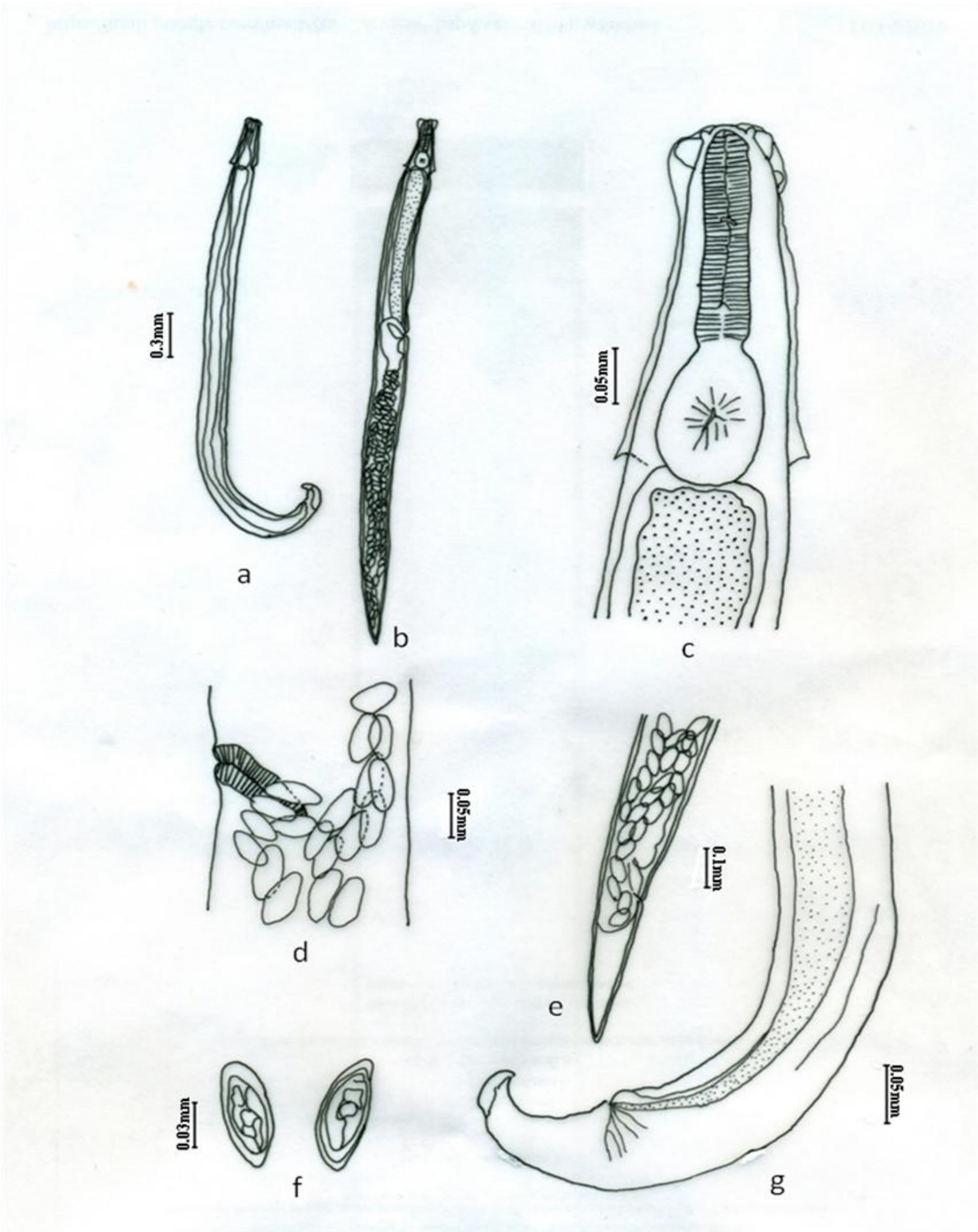


Fig. 1.13(A). *Aspiculuris* (*Paraspiculuris*) *pakistanica*

a. Whole worm, male
b. Whole worm, female
c. Anterior region showing club shaped oesophagus and cervical alae
d. Vulva opening of female
e. Posterior end of female
f. Eggs magnified
g. Posterior end of male

Fig. 1.13(B). *A* (*P*) *pakistanica*- Photomicrographs

i. ii. Anterior, posterior ends – female

iii. Posterior end of female showing the anal opening (arrow)

iv. posterior end- male

NEMATODA: Trichostrongyloidea

Order: Strongylida

Superfamily: Trichostrongyloidea

Family: Heligmonellidae (Skrjabin and Schikhobalova, 1952) Durette-Desset and Chabaud, 1971

Genus: *Nippostrongylus* Lane, 1923

Nippostrongylus brasiliensis (Travassos, 1914) Lane, 1923

(Fig. 1.14)

The collection comprised 992 specimens of this species which were collected from 7 different hosts**.**

Characters (based on the measurements of 10 females and 10 males)**:**

Male: Cephalic vesicle present; Bursal rays present with two large lateral lobes and small dorsal lobe; testes occupies anterior half of body; spicules slightly unequal.

Female: Female larger than male; vulva posterior in position; opens in front of anus; ovary filled with developing ocyte; uterus occupies posterior part of body connected to muscular ovijector leading to vagina; eggs ellipsoidal with thin shell.

Morphometric measurement of *Nippostrongylus brasiliensis* are given in Table 1.13.

Host: *R. rattus, R. nitidus, R. norvegicus, B. mackenziei, B. bowersi, B. bengalensis* and *M. Musculus*

Location: Intestine

Locality: Hlimen, Darlawn, Lungleng, Sawleng (Mizoram)

Remarks: The worms are very small in size, blood red in colour when collected from the intestine of the rodent hosts. *N. brasiliensis* is a very common intestinal parasite of rodents worldwide, and is also used as a common test parasite.

The present study provides the first report of the occurrence of this species from Mizoram, Northeast India. *R. nitidus, B. mackenziei, B. bowersi* are new hosts reported for it.

Table 1.13. Morphometric measurements of *Nippostrongylus brasiliensis*

Female		**Range**	**Mean**	**±SD**
Body length		3.20-4.419	3.807	±0.382
Maximum width at middle of body		0.06-0.09	0.077	±0.007
Cephalic vesicle	Length	0.045-0.078	0.051	±0.009
	Breadth	0.018-0.032	0.027	±0.003
Oesophagus	Length	0.24-0.288	0.266	±0.019
Eggs	Length	0.045-0.053	0.046	±0.006
	Breadth	0.017-0.029	0.026	±0.004
Cuticular Striations	width	0.018		
Excretory pore from anterior end		0.0177		
Male				
Body length		1.842-2.787	2.31	±0.344
Maximum width at middle of body		0.069-0.075	0.074	±0.002
Cephalic vesicle	Length	0.042-.0.075	0.05	±0.011
	Breadth	0.018-0.027	0.024	±0.003
Oesophagus	Length	0.092-0.12	0.106	±0.011
Spicules	Length	0.399-0.447	0.431	±0.02
	Breadth	0.39-0.438	0.419	±0.017

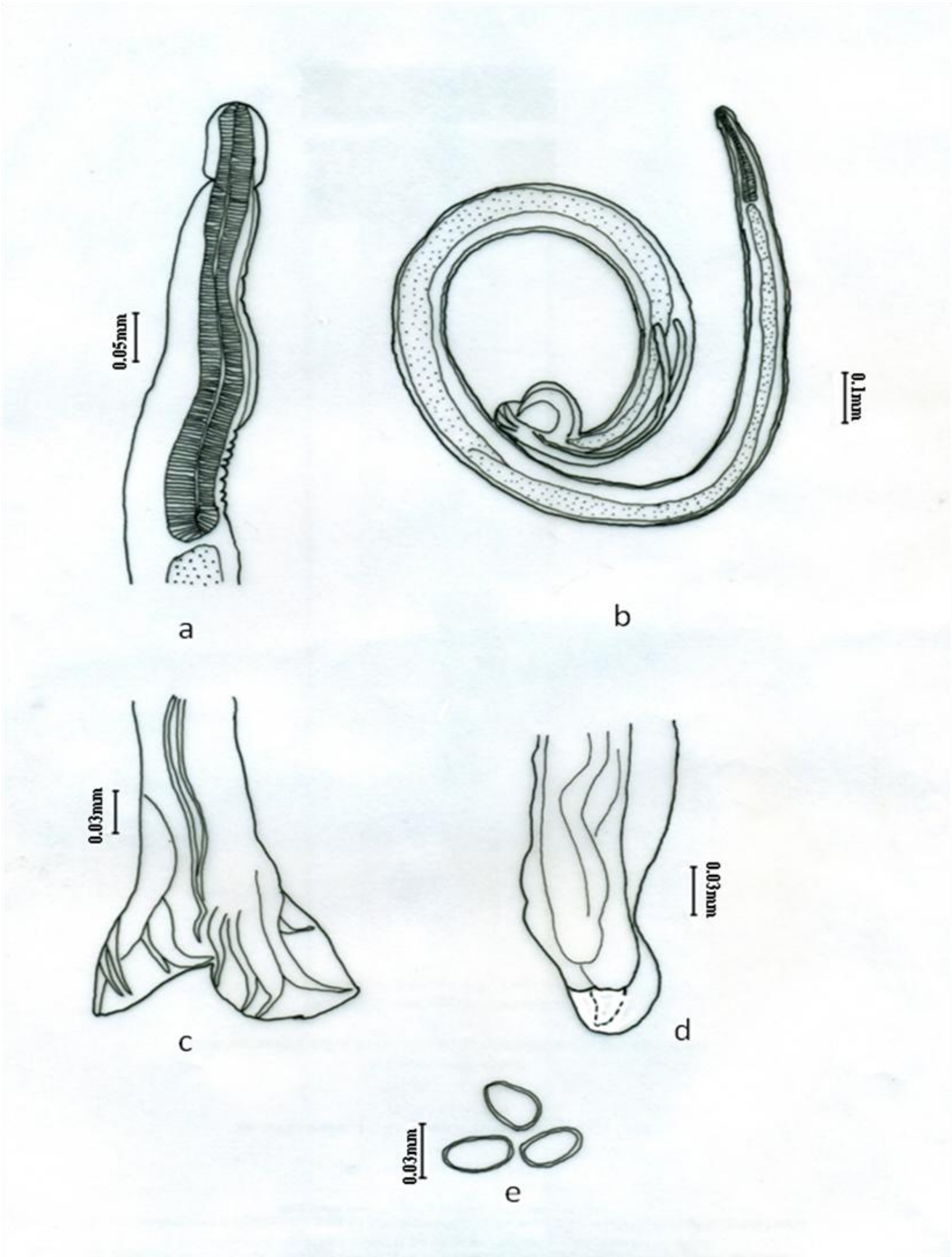


Fig. 1.14(A). *Nippostrongylus brasiliensis*

a. Anterior end showing cephalic vesicle and oesophagus
b. Whole worm of male
c. Posterior end of male showing bursal rays and slender spicules
d. Posterior end of female
e. Eggs magnified

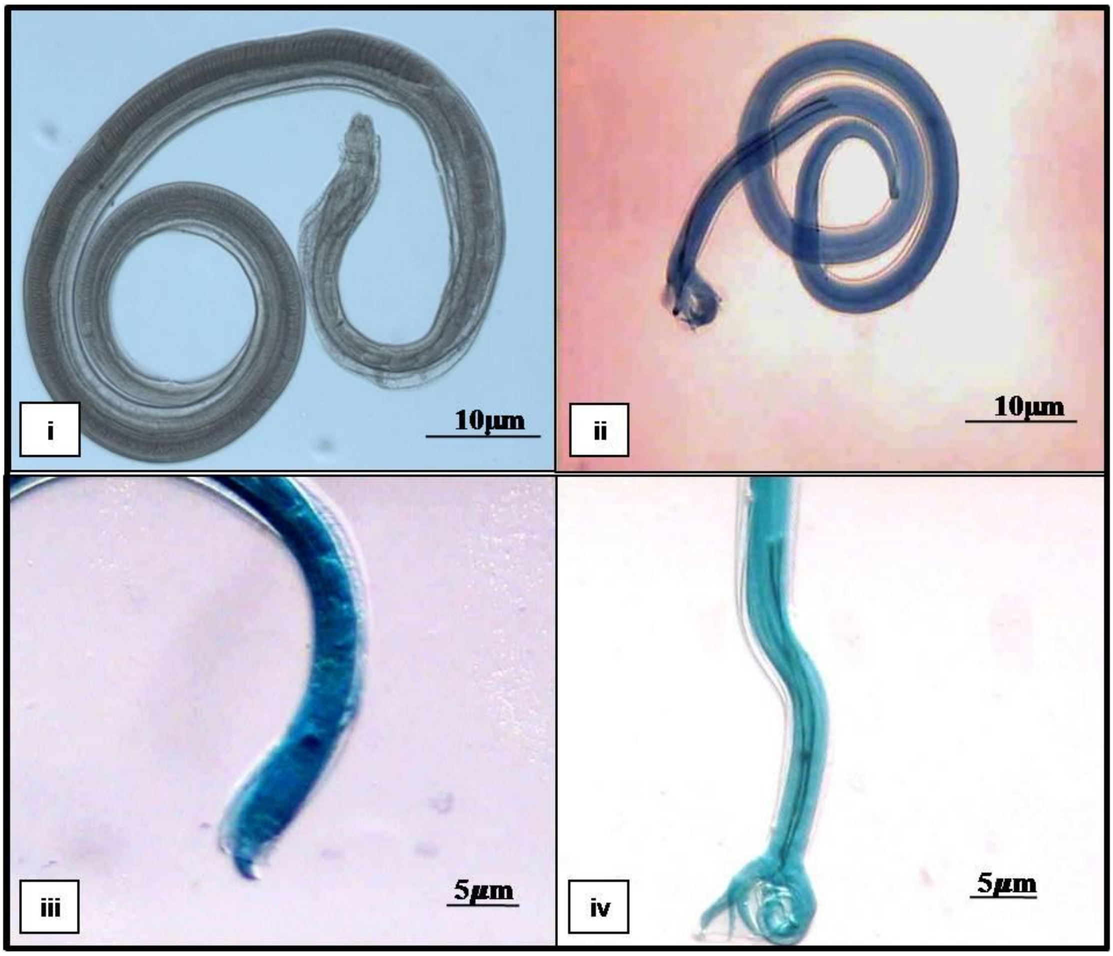


Fig. 1.14(B). *N. brasiliensis*- Photomicrographs

i. Whole worm- female

ii. Whole worm- male

iii. Posterior end of female

iv. Posterior end of male showing bursal rays and spicules

Family: Molineidae (Skrjabin and Schulz, 1937) Durette-Desset and Chabaud, 1977
Subfamily: Molininae Skrjabin and Schulz, 1937
Genus: *Hepatojarakus* Yeh, 1955

Hepatojarakus bandicoti Sood and Parshad, 1973

(Fig. 1.15)

The collection comprised a total of 22 specimens of this species recovered from 6 different hosts**.**

Description (based on measurements of 7 female and 2 male specimens)**:**

Body cylindrical; mouth or oral opening directed straight forward without lips; cephalic inflation present with transverse striation; oesophagus club shaped, without posterior bulb.

Female: Vulva post-equatorial; tail conical, long, terminating in a spike; two ovaries; vagina short; ovijector opposed; eggs oval.

Male: Well developed bursa with minute bosses on inner surface, consisting of symmetrical lateral lobes and rudimentary dorsal lobe, dorsal rays divided terminally into two short bidigitate processes; spicules short, slightly unequal, knob-like proximal end, distal end with two dissimilar processes; gubernaculum spatula shaped.

Morphometric measurements of *H. bandicoti* are given in Table 1.14.

Host: *R. rattus*, *R. nitidus, B. mackenziei, B. bengalensis*, *M. musculus*, *N. fulvescen.*
Location: Bile passages of the liver,
Locality: Hlimen, Darlawn, Bilkhawthlir, Sawleng

Remarks: The genus *Hepatojarakus* was originally described by Yeh, (1955) with *H. malayae* as the type species from the bile passages of the liver of *R. rattus jarak* in Pulanjarak, straits of Malacea. In India, Sood and Parshad (1973) reported from *H. bandicoti* from *B. bengalensis, Millardia meltada, Tatera indica* and *Mus musculus bactrianus* from Ludhiana.

The present study provides the first report of *H. bandicoti* from Mizoram, Northeast India and *R. nitidus, B. mackenziei* and *N. fulvescen* as its new host records.

Table 1.14. Morphometric measurements of *Hepatojarakus bandicoti*

Female		**Range**	**Mean**	**±SD**
Body length		13.59-23	19.72	3.299
Body width at level of middle region		0.276-0.46	0.381	±0.074
Width at anterior end		0.046-0.066	0.055	±0.006
Cephalic vesicle	Length	0.04-0.062	0.054	±0.009
	Breadth	0.048-0.068	0.056	±0.009
Oesophagus	Length	0.345-0.396	0.377	±0.021
	Breadth	0.042-0.051	0.046	±0.002
Nerve ring from anterior end		0.086-0.195	0.136	±0.05
Excretory pore from anterior end		0.225-0.234	0.229	±0.006
Vulva from posterior end		3.795-6.095	4.891	±0.83
Eggs	Length	0.034-0.06	0.05	±0.008
	Breadth	0.026-0.034	0.03	±0.003
Anal opening from posterior end		0.13-0.22	0.185	±0.03
Tip of tail		0.014-0.02	0.016	±0.002
Male				
Full length of the worm		7.82-11.47	9.645	±2.5809
Body width at level of middle region		0.23-0.276	0.253	±0.032
Width at anterior end		0.042-0.046	0.044	±0.002
Oesophagus	Length	0.282-0.315	0.298	±0.023
	Breadth	0.036-0.039	0.037	±0.002
Nerve ring from anterior end		0.105-0.18	0.142	±0.053
Cephalic vesicle	Length	0.05-0.052	0.051	±0.001
	Breadth	0.044-0.05	0.047	±0.004
Spicules Left	Length	0.162-0.196	0.179	±0.024
	Breadth	0.022-0.026	0.024	±0.002
Right	Length	0.164-0.198	0.181	±0.024
	Breadth		0.024	0
Gubernaculum	Length	0.06-0.11	0.085	±0.035
	Breadth	0.008-0.01	0.009	±0.001

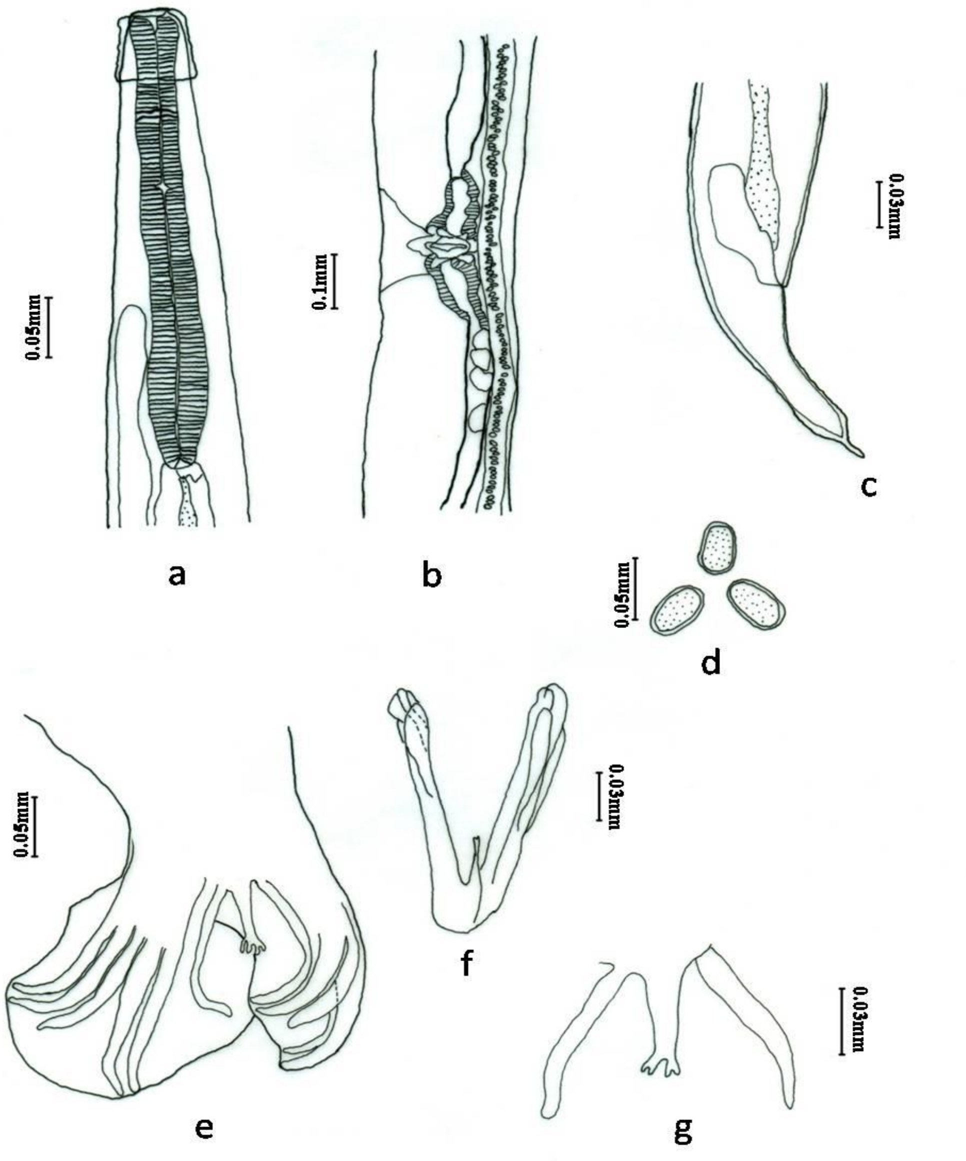


Fig. 1.15(A). *Hepatojarakus bandicoti*

a. Anterior end showing cephalic vesicle and oesophagus
b. vulva opening
c. Posterior end of female showing spike
d. Eggs magnified
e. Bursal rays
f. Spicules and gubernaculum
g. Externodorsal and dorsal rays

Fig. 1.14(B). *H. bandicoti*- Photomicrographs

i. Anterior end

ii. Eggs in uterus

iii. Posterior end of female showing the anal opening (arrow)

v. Posterior end of female with terminal spike

v. Posterior end of male

Prevalence

Nine species of rodents *viz., Cannomys badius, Berylmys bowersi, B. mackenziei, Bandicota bengalensis, Niviventer fulvescence, Rattus rattus, R. nitidus, R. norvegicus and Mus musculus,* were examined for the occurrence of parasites. The survey results of the present study revealed the presence of 9 nematodes, 4 cestode (cyclophyllidean group) and one acanthocephalan species in the parasite spectrum of the rodent hosts in the study area, whereas the trematode parasites were conspicuous by their absence. The distribution data of the parasites recovered are presented in Table 1.15 -1.16 and Fig. 1.16-1.19.

The cyclophyllidean cestode *Hymenolepis diminuta* showed highest prevalence in *R. norvegicus* (60%) and least in *R. nitidus* (15.6%); no cestode was recovered from *M. musculus. Rodentolepis* sp. was found to be most prevalent in *R. norvegicus* (30%) with its highest mean intensity (7) and abundance (0.47) recorded in *N. fulvescence. Raillietina celebensis* was collected from four species of rodents and showed maximum prevalence (6.67%) and abundance (0.13) in *N. fulvescence,* with highest mean intensity (3) in *R. nitidus.* The metacestode, *Cysticercus fasciolaris,* recovered from the liver showed maximum prevalence in *B. mackenziei* (40.9%), with highest mean intensity (3.34) in *R. nitidus* and highest abundance (0.7) in *R. norvegicus.*

Only one acanthocephalan, *Moniliformis moniliformis* (Family Moniliformidae), was recovered from a single *R. nitidus* specimen.

Among the adenophorean nematodes, *Trichuris muris* was collected only from *R. rattus* and *R. nitidus,* with highest prevalence, abundance and mean intensity found in the latter (6.25%, 2.5, and 0.15, respectively). *C. hepatica* was found to be most prevalent in *R. nitidus* (40.1%). The *C. hepatica* infection was always evident by the presence of thin narrow white streaks abounding and spreading all over the liver tissue. Because of the worms being very fragile and entangled within the liver tissue, it was very difficult to recover intact whole worms. The intensity of this infection was always found to be high with numerous parasites spread all over the organ. *Trichosomoides crassicauda,* collected from the urinary bladder of rats, showed highest prevalence (16.6%) in *Berylmys bowersi*, while its highest mean intensity (6.67) and abundance (1) were recorded in *B. bengalensis*.

The secernentean nematode, *Heterakis spumosa* showed maximum prevalence (25%), mean intensity (17.8), and abundance (4.5) in *B. bengalensis*. *Rictularia* sp., collected from both *R. rattus* and *R. nitidus*, showed higher prevalence in the former (4.49%), though more abundance (5) and mean intensity (0.15) in *R. nitidus*. The oxyurid nematode, *Syphacia obvelata* showed highest prevalence in *R. nitidus* (9.37%), while its mean intensity and abundance were highest in *M. musculus* (13.5, 1.2). *Aspiculuris* (*Paraspiculuris*) *pakistanica* was recorded to occur only in *M. musculus* and *B. mackenziei*, with higher prevalence in the latter (4.54%); however, its abundance (56) and mean intensity (1.27) were greater in *M. musculus*. *Nippostrongylus brasiliensis* was found to be most prevalent in *R. nitidus* (23.43%) with its abundance being highest (4.93) in *Mus musculus* and mean intensity, highest in *B. bengalensis* (45). The nematode *Hepatojarakus bandicoti* was collected from the bile passage and liver parenchyma of the host and showed highest prevalence in *R. rattus* (7.95%) but with maximum mean intensity (1.8) and abundance in *R. nitidus* (0.14).

In overall analysis of the results it emerged that among all the host species *R. rattus* harboured the widest range of helminth parasites with the presence of 8 nematode and 4 cestode species followed by *R. nitidus*, in which 8 nematode, 3 cestode and one acanthocephalan species were encountered.

Table 1.15: Distribution of cestodes and Acanthocephala in rodents hosts

Group of Helminth	Hosts	No. of host examined	No. of hosts infected	Prevalence (%)	Range of Intensity	Mean Intensity	Abundance
Cestoda: Cyclophyllidea:							
Hymenolepis diminuta	*Rattus rattus*	**88**	**28**	**31.8**	**2-10**	**4.64**	**1.47**
	R.nitidus	**64**	**10**	**15.6**	**2-19**	**4**	**0.62**
	R.norvegicus	**10**	**6**	**60**	**5-18**	**9.1**	**5.5**
	B. mackenziei	**22**	**8**	**36.37**	**2-5**	**2.8**	**1.04**
	B.bowersi	**12**	**4**	**33.34**	**1-5**	**1.84**	**0.94**
	B. bengalensis	**20**	**5**	**25**	**1-8**	**3.2**	**0.8**
	N. fulvescens	**15**	**4**	**26.67**	**3-10**	**7.25**	**1.94**
	C. badius	**5**	**1**	**20**	**5**	**5**	**1**
Rodentolepis sp.	*R. rattus*	**88**	**2**	**2.28**	**2-5**	**3.5**	**0.07**
	R.norvegicus	**10**	**3**	**30**	**1-7**	**3**	**0.3**
	B.bengalensis	**20**	**2**	**10**	**2-3**	**2.5**	**0.25**
	N. fulvescens	**15**	**1**	**6.67**	**4**	**7**	**0.47**
	M. musculus	**44**	**1**	**2.28**	**5**	**5**	**0.11**
Raillietina celebensis	*R. rattus*	**88**	**2**	**2.27**	**1-2**	**1.5**	**0.03**
	R.nitidus	**64**	**2**	**3.12**	**2-4**	**3**	**0.09**
	B.bengalensis	**20**	**1**	**5**	**2**	**2**	**0.1**
	N.fulvescens	**15**	**1**	**6.67**	**2**	**2**	**0.13**
Cysticercus fasciolaris	*R.rattus*	**88**	**26**	**29.5**	**1-3**	**1.3**	**0.38**
	R.nitidus	**64**	**12**	**18.75**	**1-12**	**3.34**	**0.62**
	R.norvegicus	**10**	**4**	**40**	**1-4**	**1.75**	**0. 7**
	B. mackenziei	**22**	**9**	**40.9**	**1-3**	**1.34**	**0.54**
	B.bowersi	**12**	**2**	**16.67**	**1**	**1**	**0.16**
	B.bengalensis	**20**	**6**	**30**	**1**	**1.16**	**0.35**
	N.fulvescens	**15**	**1**	**6.67**	**1**	**1**	**0.06**
	M.musculus	**44**	**2**	**4.54**	**1**	**1**	**0.04**
Acanthocephala							
Moniliformis moniliformis	*R.nitidus*	**64**	**1**	**1.56**	**15**	**15**	**0.23**

Table. 1.16. Distribution of nematodes in rodents host

Group of Helminth	Hosts	No. of host examined	No. of hosts infected	Prevalence (%)	Range of Intensity	Mean Intensity	Abundance
Nematoda: Adenophorea:							
Trichuroidea:							
Trichuris muris	*Rattus rattus*	**88**	**1**	**1.13**	**1**	**1**	**0.01**
	R.nitidus	**64**	**4**	**6.25**	**1-4**	**2.5**	**0.15**
Capillaria hepatica	*Rattus rattus*	**88**	**26**	**29.54**	**-**	**-**	**-**
	R.nitidus	**64**	**26**	**40.1**	**-**	**-**	**-**
	B.mackenziei	**22**	**7**	**31.8**	**-**	**-**	**-**
	B.bowersi	**12**	**2**	**16.6**	**-**	**-**	**-**
	N.fulvescens	**15**	**6**	**40**	**-**	**-**	**-**
	M.musculus	**44**	**1**	**2.27**	**-**	**-**	**-**
Trichosomoides crassicauda	*Rattus rattus*	**88**	**5**	**5.68**	**1-6**	**3.2**	**0.18**
	R.nitidus	**64**	**1**	**1.56**	**1**	**1**	**0.01**
	R.norvegicus	**10**	**1**	**10**	**3**	**3**	**0.3**
	B.mackenziei	**22**	**2**	**9.09**	**1**	**1**	**0.09**
	B.bowersi	**12**	**2**	**16.6**	**1-2**	**1.5**	**0.25**
	B.bengalensis	**20**	**3**	**15**	**3-10**	**6.66**	**1**
	N. Fulvescens	**15**	**1**	**6.67**	**1**	**1**	**0.06**
Secernentea:							
Heterakoidea:							
Heterakis spumosa	*Rattus rattus*	**88**	**7**	**7.95**	**1-15**	**4.85**	**0.38**
	R.nitidus	**64**	**5**	**7.81**	**1-7**	**2.8**	**0.21**
	B.mackenziei	**22**	**1**	**4.54**	**2**	**2**	**0.09**
	B.Bengalensis	**20**	**5**	**25**	**1-43**	**17.8**	**4.45**
Rictularioidea:							
Rictularia sp	*Rattus rattus*	**88**	**4**	**4.49**	**1-3**	**1**	**0.04**
	R.Nitidus	**64**	**2**	**3.12**	**2-8**	**5**	**0.15**
Oxyuroidea:							
Syphacia obvelata	*Rattus rattus*	**88**	**7**	**7.95**	**2-25**	**6.1**	**0.48**
	R.nitidus	**64**	**6**	**9.37**	**2-8**	**4.67**	**0.43**
	B.mackenziei	**22**	**1**	**4.54**	**2**	**7**	**0.31**
	M.Musculus	**44**	**4**	**9.09**	**2-6**	**13.5**	**1.2**
Aspicularis (*paraspiculuris*) *pakistanica*	*B.mackenziei*	**22**	**1**	**4.54**	**5**	**3**	**0.13**
	M.Musculus	**44**	**1**	**2.27**	**56**	**56**	**1.27**
Trichostrongyloidea:							
Nippostrongylus brasiliensis	*Rattus rattus*	**88**	**15**	**17.04**	**3-117**	**27.14**	**4.62**
	R.nitidus`	**64**	**15**	**23.43**	**2-55**	**17**	**3.98**
	R.norvegicus	**10**	**1**	**10**	**5**	**5**	**0.5**
	B.mackenziei	**22**	**4**	**18.18**	**2-20**	**10.5**	**1.9**
	B.bowersi	**12**	**1**	**8.34**	**10**	**10**	**0.84**
	B.bengalensis	**20**	**1**	**5**	**45**	**45**	**2.25**
	M.Musculus	**44**	**7**	**15.9**	**20-45**	**31**	**4.93**
Hepatojarakus bandicoti	*Rattus rattus*	**88**	**7**	**7.95**	**1**	**1.57**	**0.12**
	R.nitidus	**64**	**5**	**7.81**	**1-2**	**1.8**	**0.14**
	B.mackenziei	**22**	**1**	**4.54**	**1**	**1**	**0.04**
	B.bengalensis	**20**	**1**	**5**	**1**	**1**	**0.05**
	M.musculus	**44**	**1**	**2.27**	**1**	**1**	**0.02**
	N. Fulvescens	**15**	**1**	**6.67**	**1**	**1**	**0.06**

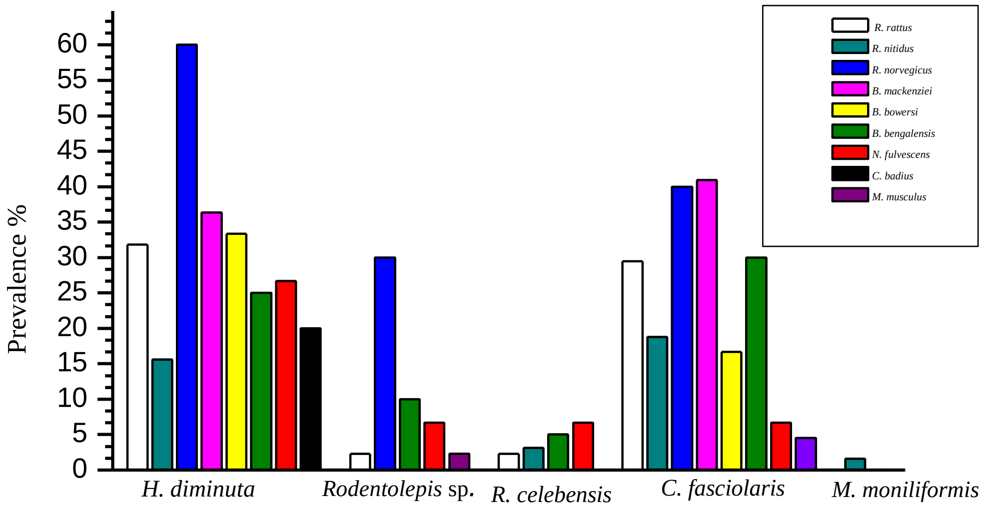


Fig. 1.16. Prevalence of cestodes and Acanthocephala in rodent hosts

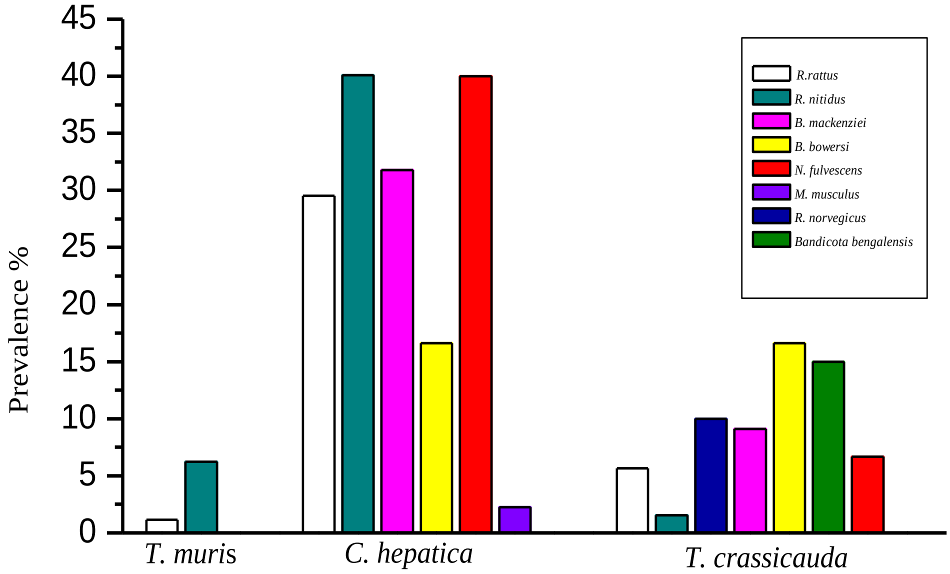


Fig. 1.17. Prevalence of adenophorean nematodes in rodent hosts

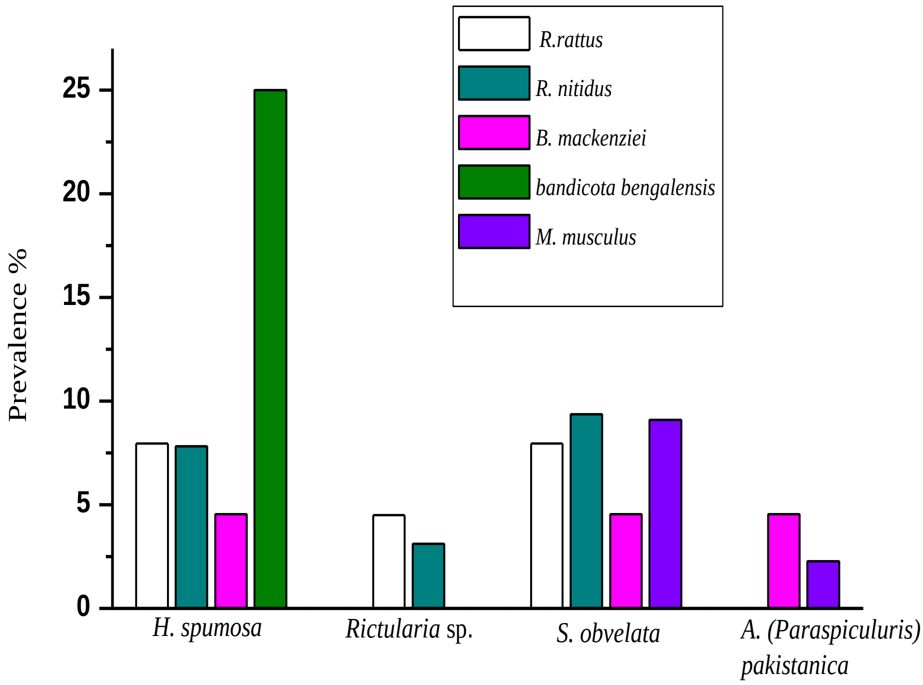


Fig. 1.18. Prevalence of secernentean nematodes in rodent hosts

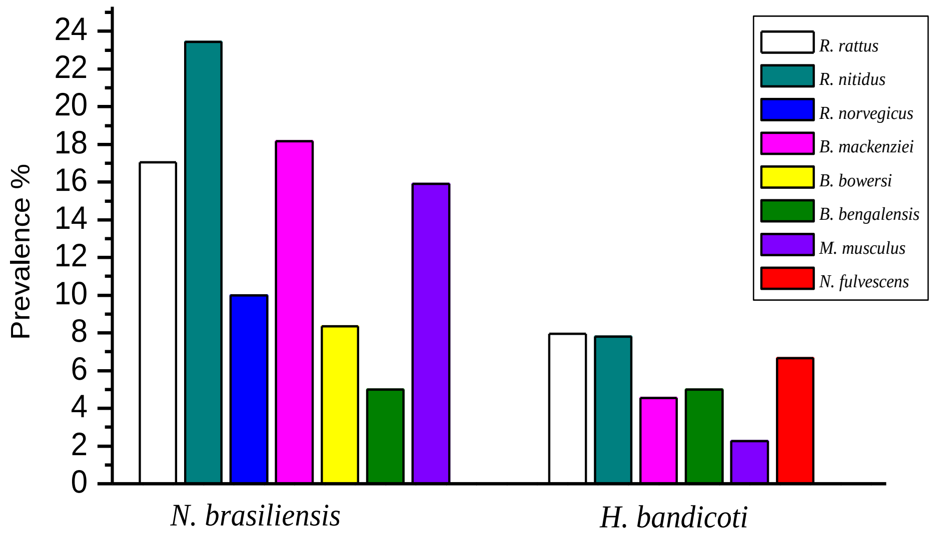


Fig. 1.19. Prevalence of trichostrongylid nematodes in rodent hosts

Studies on the sex- wise prevalence of the parasites (Table 1.17 and Fig.1.20) revealed that the prevalence of cestodes was higher in female as compared to the male hosts in case of all the rodents species excepting *M. musculus*, in which the prevalence was higher in the male hosts and in *B. bowersi* it seemed to be the same for both the sexes. Similar was the case for nematode infection where prevalence of infection was higher in the female hosts excepting *B. bowersi* and *M. musculus*, in which the prevalence of infection was higher in the male hosts. Overall the prevalence of nematodes was higher than that of cestodes in both sexes except in *R. norvegicus* in which the prevalence of cestodes was higher in both male and female hosts.

Table. 1.17. Sex-wise distribution of parasites in rodents hosts

Host	Sex	No. of host examined	No. of host infected with Cestodes	Prevalence (%)	No. of host infected with Nematodes	Prevalence (%)
R. rattus	**Male**	**47**	**20**	**42.55**	**32**	**68.08**
	Female	**41**	**21**	**51.21**	**30**	**73.17**
R. nitidus	**Male**	**28**	**7**	**25**	**17**	**60.71**
	Female	**36**	**15**	**41.67**	**27**	**75**
R. norvegicus	**Male**	**6**	**5**	**83.34**	**1**	**16.67**
	Female	**4**	**4**	**100**	**1**	**25**
B. mackenziei	**Male**	**8**	**3**	**37.5**	**5**	**62.5**
	Female	**14**	**7**	**50**	**10**	**71.42**
B. bowersi	**Male**	**6**	**2**	**33.34**	**5**	**83.34**
	Female	**6**	**2**	**33.34**	**4**	**66.67**
B. bengalensis	**Male**	**10**	**3**	**30**	**2**	**20**
	Female	**10**	**6**	**60**	**4**	**40**
N. fulvescens	**Male**	**7**	**1**	**14.28**	**5**	**71.42**
	Female	**8**	**4**	**50**	**6**	**75**
M. musculus	**Male**	**22**	**3**	**13.63**	**8**	**36.37**
	Female	**22**	**1**	**4.54**	**7**	**31.81**
C. badius	**Male**	**2**	**1**	**50**	**1**	**50**
	Female	**3**	**-**	**-**	**2**	**66.67**

In analysis of locality- wise occurrence of infection, the prevalence of cestode infection was much higher in case of the hosts collected from urban areas, excepting *M. musculus* in which higher infection of cestodes was recorded from rural areas. In contrast, the nematode infection seemed to be higher in rural areas in majority of the rodent hosts collected with the exception of *B. mackenziei*, *B. bengalensis* and *M. musculus,* all of which showed higher infection with nematodes in urban areas (Table 1.18 and Fig 1.21).

Table. 1.18. Habitat- wise distribution of parasites in rodents hosts

Host	locality	No. of host examined	No. of host infected with Cestodes	Prevalence (%)	No. of host infected with Nematodes	Prevalence (%)
R. rattus	**Rural**	**36**	**9**	**25**	**30**	**83.34**
	Urban	**52**	**32**	**61.53**	**23**	**44.23**
R. nitidus	**Rural**	**34**	**4**	**11.76**	**23**	**67.64**
	Urban	**30**	**17**	**56.67**	**20**	**66.67**
R. norvegicus	**Rural**	**-**	**-**	**-**	**-**	**20**
	Urban	**10**	**9**	**90**	**2**	**50**
B. mackenziei	**Rural**	**14**	**4**	**28.57**	**7**	**100**
	Urban	**8**	**7**	**87.5**	**8**	**80**
B. bowersi	**Rural**	**5**	**1**	**20**	**4**	**71.42**
	Urban	**7**	**3**	**42.85**	**5**	**11.12**
B. bengalensis	**Rural**	**9**	**3**	**33.34**	**1**	**45.46**
	Urban	**11**	**7**	**63.64**	**5**	**88.89**
N. fulvescens	**Rural**	**9**	**1**	**11.12**	**8**	**50**
	Urban	**6**	**4**	**66.67**	**3**	**25**
M. musculus	**Rural**	**8**	**1**	**12.5**	**2**	**36.12**
	Urban	**36**	**2**	**5.56**	**13**	**60**
C. badius	**Rural**	**5**	**1**	**20**	**3**	**-**
	Urban	**-**	**-**	**-**	**-**	

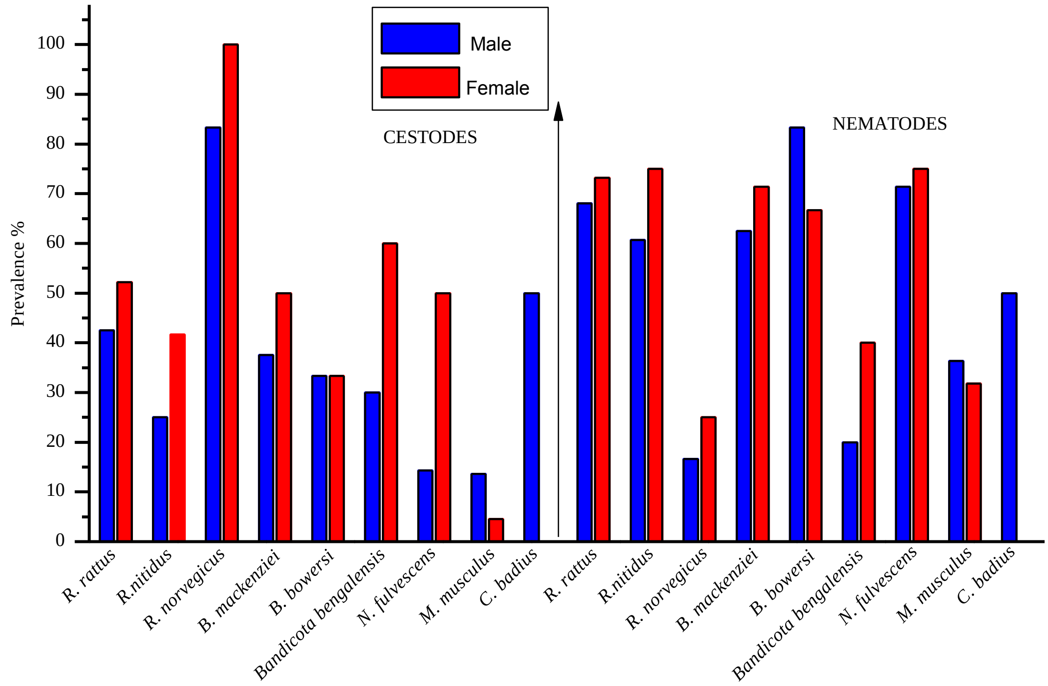


Fig. 1.20. Sex-wise Prevalence of helminth parasites in rodent hosts

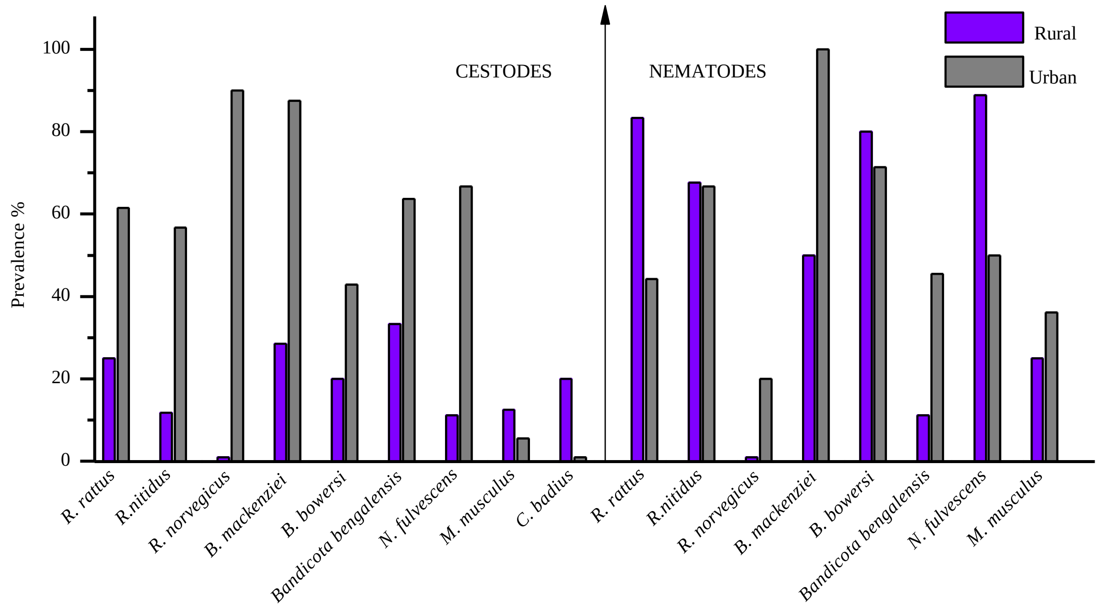


Fig. 1.21. Habitat- wise prevalence of helminth parasites in rodent hosts

DISCUSSION

The present study gives the first overview on the parasitic fauna of rodents in bamboo-rich state of Mizoram, reporting the presence of 14 species of helminths from 9 different species of hosts. In India, various workers have reported a wide range of parasites from different rodent hosts. A complete checklist of nematode parasites of Indian rodents is also available (Sood and Parshad, 1981). A species of *Hepatojarakus bandicoti* which was collected from the biliary passages of the liver and the liver parenchyma in the present study was described by Sood and Parshad (1973) from four different rodent hosts, viz. *Bandicota bengalensis*, *Millardia meltada*, *Tatera indica* and *Mus musculus batrianus*. Johnson (1969a) also reported *Rictularia* sp. from *R. rattus, Funambulus pennati* and *Felis catus* from Jodhpur, as has also been reported in the present study. In the same year Johnson (1969b) also described and reported a nematode of the genus *Aspiculuris* from the common house rat *R. rattus*. Nama and Parihar (1976) reported 8 helminth species from *R. rattus rufescens.* Saxena and Nama (1977) reported the occurrence of the genus *Aspiculuris* as the predominant nematode parasite among the common house rats in Johdpur. This report is in contrast with the present findings that show a low prevalence of *Aspiculuris* infection in the study area. Balachandra and Ranade (1978) reported the occurrence of 7 helminth species in *R. rattus* and *B. indica* in Poona city, of which *H. diminuta* was one of the predominant species collected. The present observations are in confirmity with this report.

Singhvi and Johnson (1979) also worked on the population dynamics of nematode parasites of house rat. Gupta and Singla (1979) also reported the presence of *H. diminuta* and gave a full description of the parasite based on a single specimen from the intestine of a field rat, *Mus terricolor.* In the present study *H. diminuta* is the most abundant cestode reported from all the rodent species collected except from *M. musculus*. Malhotra and Capoor (1987) also reported the occurrence of hymenolepid species in Indian rats. Johnson et al. (1987) reported the presence of 9 helminth species in *R. rattus,* of which 6 were also reported in the present study. Singla et al. (2008) reported the occurrence of 5 helminth species from 3 different species of rodents, of which four (*T. taeniaeformis*, *H. diminuta*, *Trichuris muris* and *M. moniliformis*) have also been reported in the present study. The helminth parasite diversity in *Rattus* spp. collected from wet market of Manila (Philippines) also included the species of *Hymenolepis*, *Taenia*, *Capillaria*, *Trichosomoides* and *Moniliformis* as reported in rats of Mizoram (Claveria *et al*., 2005). However the wild rodents from the Endau Rompin National Park (Malaysia) were found to harbour a rich worm diversity that included 23 nematode, 3 cestode and 2 trematode species and in addition one pentastomide species (Syed-Arnez and Zain, 2006).

The occurrence of high prevalence of cestodes in urban areas and the occurence of a wide range of parasites in *R. rattus* and *R. nitidus* as revealed in the present survey, may be attributed to the fact that both these rodents species were collected mostly in proximity to human dwellings where chances of contact with the intermediate host such as grain beetle and other insects is very high. Endoparasitic infection is related with the availability of the intermediate hosts. (Flynn, 1973; Khan, 1990; Rafique et al., 2009). In contrast to *Rattus* spp, the rodent *Cannomys badius,* which was always collected directly from the bamboo forests and surrounding areas far from human habitations, harboured a low range and minimum parasite load. However, interestingly the cestode parasites, need an insects intermediate host and which were commonly found in urban areas were also recovered from *C. badius*, even though this rodent was collected only from rural areas.

Many theories have suggested that males are more susceptible to the parasitic infections than female (Ferrari et al., 2004). The male hormone testosterone is supposed to implicate a negative effect on the immune function, thereby predicting that male will have more parasitic infection than female (Grossman, 1989; Folstad et al., 1992). This seems to be in contrast to the present findings, as female hosts were found to have more helminth infections than male, excepting *M. musculus,* an observation which agrees with the findings of Kataranovski et al. (2008) who reported higher prevalence of infection in males in this host species.

The absence of the trematode component in the parasite spectrum may be attributed to the fact that trematodes need implication of aquatic molluscan intermediate hosts for completion of their life cycle; the terrestrial habitat of all the rodents collected might have restrained their contact with water bodies in which thrives the snail population and thus removed the possibility of such infections.

Among the helminth parasites, *Capillaria hepatica* and the metacestode of *Taenia* sp. showed a considerably high prevalence (up to 50%) in several of the host species surveyed. A very high prevalence of *Taenia* metacestode and 100% infection of *Capillaria hepatica* (both exhibiting high parasitemia) have been reported in *Rattus* spp in Philippines (Claveria et al., 2005). In view of their tissue damaging effect and common occurrence, these two parasites can be tested for their potential as biological control agents for rodent pests as also suggested by Singleton and McCallum (1990).

Chapter - 2

Molecular characterization of metacestode of *Taenia* sp. and *Hymenolepis diminuta*

INTRODUCTION

The cestode systematics have always been problematic for various reasons; difficulties have been related to poor accessibility or fragility of the specimens, substandard or incomplete taxonomic descriptions or conflicting information derived from various classes of morphological and ontogenetic characters (Brooks et al., 1991; Mariaux, 1996). Studies on the phylogeny of tapeworms (Eucestoda) have achieved considerable progress and new hypotheses based on morphological, life cycle, ultrastructural and molecular data have been proposed (Hoberg et al., 1997, 1999; Justine, 1998; Mariaux, 1998). The ribosomal DNA (rDNA) clusters have been used for genetic studies as this region is highly repeated and contains variable regions flanked by more conserved regions (Hillis and Dixon, 1991). PCR techniques that utilize the second internal transcribed spacer (ITS2) sequences (which lies between 5.8S and 28S coding regions) have proven to be a reliable tool in identifying the platyhelminth parasite species and their phylogenetic relationships (Blair et al., 1999; Scholz et al., 2004). Mitochondrial genome has also been used in taxonomic studies as it is a rapidly evolving genome as compared to nuclear DNA and has minimal non-coding DNA and no introns (Avise 1994; Mcmanus and Bowles, 1996). For the design of oligonucleotide primers which are used for amplification of variable region of the genome, conserved coding regions such as cytochrome oxidase *c* subunitI (COI) gene are ideal for studying closely related species (Mcmanus and Bowles, 1996; Blouin et al., 1998). More recently RNA secondary structures are found to be useful in systematics because they include characteristics, not found in the primary sequence, that give 'morphological' information (Caetano-Anolles, 2002). The novel approach of molecular morphometrics that relies both on traditional morphological comparison and comparative molecular sequence analysis by measuring the structural parameters of the ITS2 secondary structure homologies (geometrical features, bond energies, base composition etc.) is recently being used to study the phylogenetic relationships of various species (Billoud et al., 2000). This method allows one to take into account the regions where multiple alignments are barely reliable because of a large number of insertions and deletions. This method is based on the assumption that secondary structure can be phylogenetically as significant as primary sequence. It is well known that rRNA is highly conserved throughout evolution. Thus, the secondary-structure elements of the RNA molecule, i.e., the helices, loops, bulges, and separating single-stranded portions, can be considered phylogenetic characters (Zwieb et al., 1981, Schultz et al., 2005, Grajales et al., 2007).

Cestodes of the family Taeniidae are parasites of carnivore animals and human hosts, which use mammals as their intermediate hosts where the larval stage develops in the tissues causing significant harm to the host (Eckert et al., 2005). As these parasites are of great medical and veterinary significance, several studies have been focused at the species level (Abuladze, 1964; Verster, 1969; Rausch, 1994). Approximately 40 species of the genus *Taenia* have been recognized based on morphological studies of the adult specimen (Hoberg et al., 2000). Many species have also been established for metacestodes (Murai et al., 1993), though only a few reports have been published on the phylogeny of the taeniids (Hoberg et al., 2000) Phylogenetic relationships of taeniid cestodes have been constructed using morphology, host specificity and biological traits such as asexual reproductive potential. However it is still difficult to come up with a reliable conclusion on the phylogenetic relationship not only between strains or species but also between the subfamilies of Taeniidae (Okamoto et al., 1995). Recent studies have shown high level of interspecific variations for molecular characters, which are useful for characterization of species of *Taenia* (Bowles and McManus, 1994; Gasser and Chilton, 1995; De Queiroz and Alkire, 1998; Nickisch-Rosenegk et al., 1999).

Taenia taeniaeformis is a taeniid cestode parasite, the adult form of which is found in cats and other carnivores and uses rodents as the intermediate hosts where the larval form or the cysticercus develops as a fluid filled metacestode in different organs (Hsu, 1979; Georgi and Georgi, 1990; Hanes, 1995). *Hymenolepis diminuta,* is a cosmopolitan parasite, which has been commonly reported from the intestine of rodent hosts worldwide; the intermediate hosts are arthropods in which cysticercoids develop and adults are found in birds and mammals.

During an exploratory survey of rodent pests during bamboo-flowering times in forests of Mizoram, Northeast India for their helminth parasite spectrum, the metacestodes and adult cyclophyllidean tapeworms occurring in the intestine were frequently encountered infecting the liver lobes and body cavity of the host. The present study aimed at identifying this metacestode and the adult tapeworm based on molecular characterization supplementing the morphological criteria.

MATERIALS AND METHODS

The parasites

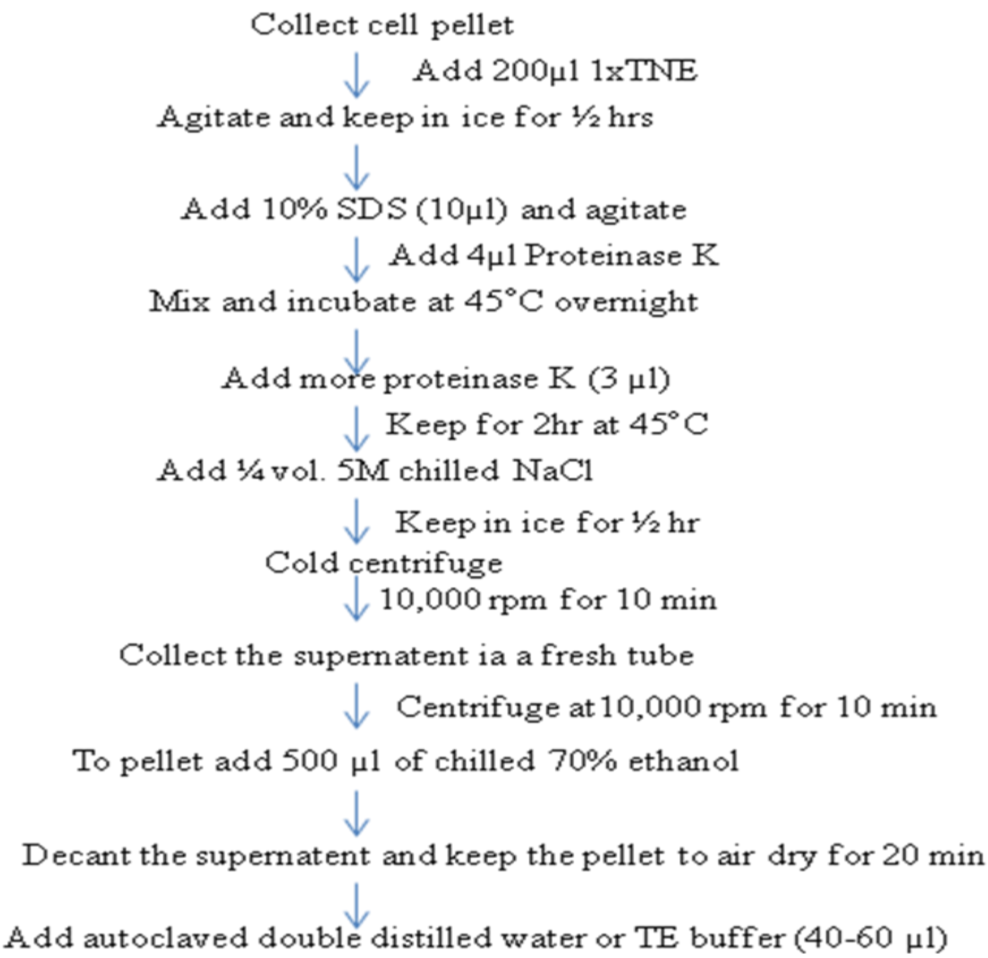
The metacestode were found encysted in the liver tissues of *Rattus rattus*, the commonly prevalent rodent species in the region. The cyst capsules were opened by making a small slit to release the parasite. The adult cestode, *Hymenolepis diminuta* was found to be inhabiting the intestinal lumen of the rodent hosts. The specimen were duly processed for whole-mount preparation and subsequent microscopy observation, while some of the fresh or 70% alcohol fixed specimen were processed for DNA extraction and PCR amplification.

Molecular characterization

DNA extraction

The genomic DNA of the parasite was isolated using standard ethanol precipitation technique (Sambrooke et al., 1989) as detailed below (Flowchart 1):

Flowchart 1. DNA extraction from parasites - Ethanol precipitation technique



The rDNA region spanning the ITS1, ITS2 and mitochondrial COI were amplified by PCR. The primers used were designed based on the conserved sequences of *Schistosoma* spp and are considered to be the universal primers for trematode species (Bowles et al., 1995).

Primers

ITS1 BD1 (Forward): 5'- GTCGTAACAAGGTTTCCGTA-3'

4S (Reverse): 5'- TCTAGATGCGTTCGAA (G/A) TGTCGATG-3'

ITS2 3S (Forward): 5'- GGTACCGGTGGATCACTCGGCTCGTG-3'

A28 (Reverse): 5'- GGGATCCTGGTTAGTTTCTTTTCCTCCGC-3'.

CO1 JB3 (forward): 5’-TTTTTTGGGCATCCTGAGGTTTAT-3’

JB4 (reverse): 5’-TAAAGAAAGAACATAATGAAAATG-3’.

PCR amplification

PCR amplification was done following standard procedure (White, 1993) with minor modifications in 100mM Tris-HCl (pH 9.0), 500mM KCl, 1.5 mM $MgCl^2$ and 0.2 mM deoxynucleotide triphosphates (each of deoxyadenosine triphosphate, deoxyguanosine triphosphate, deoxycytidine triphosphate, deoxythymidine triphosphate), 0.25 mM of each primer and 2.5 U of *Taq* polymerase (Bangalore Genei, India) (Tandon et al., 2007). The PCR cocktail (final reaction volume 25µl) was amplified with the following conditions: (i) ITS1- Initial denaturation at 94^{o}C for 5 min, then 35 cycles including denaturation at 94^{o}C for 40 sec, annealing at 56^{o}C for 1 min, extension at 72^{o}C for 1.05 min, and final extension at 72^{o}C for 10 min; (ii) ITS2 - initial denaturation at 94^{o}C for 5 min, then 26 cycles including denaturation at 94^{o}C for 30 sec, annealing at 55^{o}C for 38 sec, extension at 72^{o}C for 42 sec, followed by final extension for 10 sec at 72^{o}C; (iii) COI region - initial denaturation at 95^{o}C for 1 min, then 35 cycles including denaturation at 94^{o}C for 1 min, annealing at 55^{o}C for 1.2 min, extension at 72^{o}C for 1.2 min, followed by final extension for 10 sec at 72^{o}C.

The resultant PCR products were separated by electrophoresis through 1.5% (w/v) agarose gel in TAE buffer, stained with ethidium bromide, transilluminated under ultraviolet light and then photographed. The known size fragments of 100 bp ladder in agarose gel were used as marker. The PCR product was purified using Genei Quick PCR Purification Kit and sequenced in both directions on an automated sequencer. The sequences were submitted to GenBank for obtaining their accession numbers.

Sequences deposited in GenBank

i) FJ939132- *Hymenolepis diminuta* adult 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.

ii) FJ939133- *Taenia taeniaeformis* 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.

iii) FJ939134- *Taenia taeniaeformis* adult internal transcribed spacer, partial sequence.

iv) FJ939135- *Taenia taeniaeformis* cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial.

Analysis of the nucleotide sequence

The sequences obtained were analysed using the various bioinformatic tools such as Basic Local Alignment Search Tools (BLAST) for similarity search (http://www.ncbi.nlm.nih.gov/blast), multiple alignment and phylogenetic prediction using Clustalw (provided at the URL http://www.ebi.ac.uk/clustalw) with default gap and extension penalties (Thompson et al., 1994).

Phylogenetic tree construction

For interpreting the results obtained, different tree building models were considered. Phylogenetic reconstructions were done using the MEGA 4.0 package; sequences arranged with MEGA format were entered in the MEGA for construction of the phylogenetic trees, which were inferred using distance- based methods such as Neighbour-Joining (NJ), minimum evolution, unweighted pair group method with arithmetic mean (UPGMA) and character-based method like Maximum Parsimony (MP) in MEGA 4.0 (Tamura et al., 2007). Only unique sequences were used in tree construction. The phylogenetic accuracy for the individual branches of the resulting tree was determined by 1000 bootstrap replications in MEGA (Hillis and Bull, 1993).

Predicted ITS2 RNA secondary structures and analysis

rRNA molacules are widely used in primary sequences and secondary structure comparisons as molecular structure are a direct source of measureable information. The secondary structure elements of RNA molecules (i.e. the helices, loops, bulges) are generally considered as phylogenetic characters owing to their evolutionary conservativeness. Most of the secondary structure folding are functionally essential despite primary sequence divergence (Wheeler and Honeycutt, 1988) and serve as phylogenetic signals.

ITS2 secondary structures of the cestodes were folded with the help of MFold (Zucker, 2003) by screening for thermodynamically optimal and suboptimal secondary structures (default settings with (T=25°C). The secondary structure in Vienna (dot-bracket-dot) format was used as an input for multiple alignment RNA (MARNA Siebert and Backofen, 2005) to calculate sequence structure multiple alignment. However, there was a limitation with the online server that the maximum length of one RNA sequence is restricted to 500 bases; hence some of the ITS sequences whose exact boundary information was available from Genbank graphics view was trimmed for facilitating MARNA to run. Some more cestode sequences were also taken whose 5.8S, 28S and ITS2 regions were clearly defined so as to include in the sequence structure multiple alignment dataset.

GC content

GC richness contributes to physical attributes of RNA secondary structures as there is a correlation between GC content and optimal growth temperatures. GC rich compound are more stable at higher temperatures then GC deficient RNA molecules. Hence, to substantiate the stability of the ITS2 secondary structures the GC content was calculated using oligo calculator available at http://www.pitt.edu/~rsup/ OligoCalc.html.

RESULTS

Metacestode

Morphological Analysis

The morphological examination of the metacestode parasite revealed typical taeniid features with four prominent lateral suckers; rostellum armed with double rows of hooks; all hooks typically taenoid type having long blunt handle with sharp pointed blade; and short or elongated and segmented strobila terminating with a bladder, thus resembling a small tapeworm but without reproductive organs. All the morphological characters are in consistence with those of *Cysticercus fasciolaris*, the adult form of which is *Taenia taeniaeformis.*

Molecular Analysis

PCR amplification of ITS and COI regions

The rDNA ITS (1, 2) and mtCOI regions of the metacestode were successfully amplified by using the primers of *Schistosoma* species as mentioned above. PCR amplification of the three region showed a single band of size 744bp, 443bp and 373bp respectively (Figs. 2.1 a, b, c).

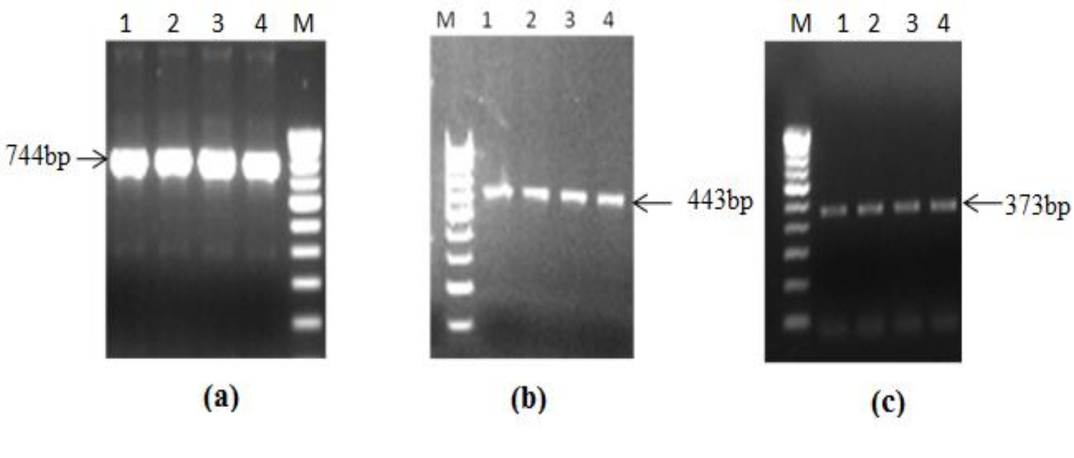


**Fig.2.1. PCR products of the metacestode- (a) ITS2 (b) ITS1 and (c) CO1 using primer set 3S-A28, BD1-4S and JB3-JB4, respectively.
Lanes 1-4 metacestode; M= marker**

The nucleotide sequences obtained for ITS2 are shown below as raw sequences:

ITS2

AAATTTTTGGTTTTTGTATTGTGTGGATGCCTGGGTGTGTGTGAGTCAGAG
GGCAGCAAAGGAGTGAATTAATTTAATACAGATTGATTTGTACATTACCC
TCTTGACTTCCATTGCGTCCATAGGCTCGCGTCTGGCCATGTCTGTGCTAG
TGTCGTATAATAAAATATCACTTAGCGTAACGTGCAGTGGCTCGGGCGAT
TCCCTTCCCTGCCCGCCTCTGGTCGTGTGCGGTGCTGTCCTGTGCGGTGCG
TTTCAGTGCATGTCATGTAGTGTAAGGTTGACGGTGCTGCCGAGGACAGG
GCAGTACAGGTCAGCTGTGCTGTCCTGTGCTGTGCTGTGCAGTGCTGTGCT
GCGTTGCGTTGCACTCCTGGTCGTCCTATCCACAAGACGGTGGATAGGGG
CTGTATGGAGTGGCGCATTGTGTGTCGCGCTTGTGCGGTAGTGGTGCTTGC
AGTTCACGGATCGTGGCCCAGTGAGTGGTGGTGTGGTGCTGGCGCGATTG
GCTTTGTCAGTTATTCGCGTCTGTAGTGCACGCGCACGTGAAGTGGTCTGC
GCGGGTGCGCCCACCTAGGCGGTTGGGCAGCGGCAGCGGCAGCGGCATT
AATAGCAGCGGCGGAGGCGCACCGTGTTTGGGTTAGGGTGTGCTGATGCA
GCATCTACAACAGCAACAGCAACAAACAGCAGTAACAACTGACCTCGGA
TTAGGCGAGAGTACCCGCTGAACATAAGCATATCAATAAT

ITS1

AGAGATATGTTTTTTTTTTGGGTGGGGGGGGGGGGTGTGTGAGCAGGGG
GGGGGTGTTTTGTCTTCCTTCTTCGCGCGCGTGTCCGTCTCTCTCTCTTTCT
GTGTGTGTGTGTGTGTGCGCGGGGGAGTGGGAGCCCGGTCCATCCCGGGG
GCGGAGGGGTTTATACACATGCGCGCGCGATTTTGTGTGTGTGTGTGCGTG
TGTGCGAGGCGTAAGATAGTGATGGATGCGACGGTTGTGCAGTTGCGTCC
GTCCGCGCCCCATCATGTGTCATGTGTTTGGTGTAGTGGTGATGGTTGTGC
GTGAGTGTAAGGCGATGTGAAGTGGAGAAGTGGAATGGCATGGCAATAA
CTGTGCGCGGTGGATCACTCGGCTCGTGTGTCGATGAAGGGTGCAGCCAA
CTGTGAGAATTAGTGTGTAGTAGCCAGAGGTGCTCTCGTA

COI

ATTCTTCCTGGATTTGGTATTATTAGACATATATGTTTAAGAATTAGTATG
TCTTCGGATGTGTTTGGTTTTTATGGTTTATTGTTTGCTATGTTTTCTATAGT
TTGTTTAGGAAGAAGGGTGTGAGGTCATCATATGTTTACTGTTGGGTTAGA
TGTAAAGACGGCTGTGTTTTTTAGCTCTATAACTATGATTATTGGAGTACC
TACAGGTATAAAGGTTTTTACATGATTGTATATGTTGTTGAATGCTCGAGT
CAAAAAGAGTGATCCTGTTTTATGATGAATTGTTTCTTTTATTATTCTGTTT
ACGTTTGGTGGGGTTACTGGTATAGTATTATCGGCTTGTGTTTTAGATAAA
GTGTTACATGATAC

The nucleotide sequences obtained from the PCR products were put to BLAST and compared with other available cyclophyllidean cestode sequences from GenBank (Table 2.1).

Table 2.1. Taeniid species and their various geographical isolates used in the study with respective GenBank accession numbers. (* Query sequence)

Name of parasites	Host	Geographical Isolate	Accession No.		
			ITS2	ITS1	CO1
Metacestode*	*Rattus rattus*	Mizoram, India	FJ 939133	FJ939134	FJ939135
Taenia taeniaeformis	Wistar rat , *Apodemus sylvaticus* , *Rattus norvegicus* , *Rattus rattus*	India , Kazakhstan, Japan	EU 051352	EU051351	EU544597, AB221484, EF090612
Taenia asiatica	PMD-3 Clone	Taiwan		AY606272	-
Taenia saginata	*Bos indicus*	China , Thailand	AY825542	AY392045	AB465239
Taenia solium	*Sus scrofa domestica*	Kolkata, India		EF090614	
Taenia crassiceps	*Mus* sp.	USA	DQ099564	-	-
Taenia serialis	*Canis latrans*	USA	DQ099574		-
Taenia hydatigena	*Canis familiaris* , *Rangifer tarandus*	Kenya, Finland	-	-	AM503316 , EU544552
T. Regis	*Panthera leo*	Kenya	-	-	AM503330
T. Twichelli	*Gulo gulo*	Russia	-	-	EU544598
T. martis	*Myodes rufocanus*	China	-	--	EU544558
T. madoquae	*Canis mesomelas*	Kenya	-	-	AM503325
T. multiceps	*Ovis aries*	Turkey	-	-	EF393620
T. krabbei	*Vulpes lagopus*	Norway	-	-	EU544579
T. polyacantha	*Lemmus trimucronatus*	Canada	-	-	EU544595
T. parva	*Apodemus sylvaticus*	Spain	-	-	EU544580
Hymenolepis diminuta	Rodents , *R. rattus*	Australia , India	FJ939132	AF461125	-
Hymenolepis nana	Rodents , *Mesocricetus auratus*	Australia , Uruguay	AB494477	AF461124	-
Hymenolepis microstoma	Laboratory rodents	Japan	AB494478	-	-
Raillietina australis	*Dromaius novahollandiae*	Australia	AY382317	-	-
Raillietina chiltoni	*Dromaius novahollandiae*	Australia	AY382319	-	-
Mesocestoides corti	*Canis familaris*	USA	AF119696	-	-
Mesocestoides sp.	*Canis familaris*	USA	AF119697	-	-
Moniezia monardi	*Capricornis crispus*	Japan	-	AB367791	-
Paranoplocephala arctica	*Dicrostomyx spp.*	Finland	-	AY299558	-
Anoplocephala perfoliata	*Equus ferus caballus*	Germany	-	AJ578151	-

Pairwise and Multiple Alignment

The BLAST hits result shows that the sequences of the metacestode are closer to those of species of *Taenia* sp. with maximum similarity to *Taenia taeniaeformis.* For pairwise alignment of ITS sequences, only *T. taeniaeformis* (Hyderabad India isolate) could be used, since there is no information available in GenBank for ITS (1,2) sequences pertaining to other cyclophyllidean or taeniid species. However, for CO1 region, sequences for three isolates were available for comparisons. In pairwise alignment of the ITS2 sequences and flanking regions of the query sequences with sequences of *Taenia taeniaeformis* (Hyderabad India isolate) showed the presence of 6.4% mismatches (Fig. 2.2).

```
Metacestode_Mizoram            GGTGCGTTTCAGTGCATGTCATGTAGTGTAAGGTTGACGGTGCTGCCGAG
T_taeniaeformis_Hyderabad      GGTGTGGTGCAGTGGCTTCCCTCTTTCGCAAGGCTGTTGCTGGTGCCCTG
                               **** * * *****  *  * * *   * **** **  * ** ****  *

Metacestode_Mizoram            GACAGGGCAGTACAGGTCA--------------------GCTGTGCTGTC
T_taeniaeformis_Hyderabad      GCTGCGGCACTACGGTGCAAGGCTTGATCCCTGTGCTCCGCTGCGCTGTG
                               *    **** *** *  **                    **** ***** 

Metacestode_Mizoram            CTGTGCTG--------------TGCTGTGCAGTGCTGTGCTGCGTTGCGT
T_taeniaeformis_Hyderabad      CTGCGCTGCGCTGCGCTGCGTGTGCTGTGCAGTGCTGTGCTGCGTTGCGT
                               *** ****              ****************************

Metacestode_Mizoram            TGCACTCCTGGTCGTCCTATCCACAAGACGGTGGATAGGGGCTGTATGGA
T_taeniaeformis_Hyderabad      TGCACTCCTGGCCGTCCTATCCACAAGACGGTGGATAGGGGCTGTATGGA
                               *********** **************************************

Metacestode_Mizoram            GTGGCGCATTGTGTGTCGCGCTTGTGCGGTAGTGGTGCTTGCAGTTCACG
T_taeniaeformis_Hyderabad      GTGGCGCATTGTGTGTCGCGCTTGTGCGGTAGTGGTGCTTGCAGTTCCCG
                               *********************************************** **

Metacestode_Mizoram            GATCGTGGCCCAGTGAGTGGTGGTGTGGTGCTGGCGCGATTGGCTTTGTC
T_taeniaeformis_Hyderabad      GATCGTGGCCCAGTGAGTGGTGGTGTGGTGCTGGCGCGATTGGCTTTGTC
                               **************************************************

Metacestode_Mizoram            AGTTATTCGCGTCTGTAGTGCACGCGCACGTGAAGTGGTCTGCGCGGGTG
T_taeniaeformis_Hyderabad      AGTTATTCGCGTCTGTAGTGCACGCGCACGTGAAGTGGTCTGCGCGGGTG
                               **************************************************

Metacestode_Mizoram            CGCCCACCTAGGCGGTTGGGCAGCGGCAGCGGCAGCGGCATTAATAGCAG
T_taeniaeformis_Hyderabad      CGCCCACCTAGGCGGTTGGGCAGCGGCA------------TTAATAGCAG
                               ****************************            **********

Metacestode_Mizoram            CGGCGGAGGCGCACCGTGTTTGGGTTAGGGTGTGCTGATGCAGCATCTAC
T_taeniaeformis_Hyderabad      CGGCGGAGGCGCACCGTGTTTGGGTTAGGGTGTGCTGATGCAGCATCTAC
                               **************************************************

Metacestode_Mizoram            AACAGCAACAGCAACAAACAGCAGTAACAACTGACCTCGGATTAGGCGAG
T_taeniaeformis_Hyderabad      AACAGCAACAGCAACAAACAGCAGTAACAACTGAC---------------
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Fig.2.2. Pairwise alignment of the query metacestode with *Taenia taeniaeformis* ITS2

However, with regard to ITS1, 14.3% mismatches were observed (Fig. 2.3.)

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Metacestode_Mizoram             ---------------------AGAGATATGTTTTTTTTTTGGGTGGGGGG
T_taeniaeformis_Hyderabad       TCTTGTGCGTGTAGGCGTGGAAGAGATGTGTAGTTGTGTTGTGGGGGTGG
                                                     ****** ***  ** * *** * *** **

Metacestode_Mizoram             GGGGGGGTGTGTGAGCAGGGGGGGGGTGTTTTGTCTTCCTTCTTCGCGCG
T_taeniaeformis_Hyderabad       GTGGTGGTGAGGGGTTCAGTGGTGTTTGGTCTTCCCTTCTCCCTTGCGCG
                                * ** **** * *     * ** *  ** * *  * * ** * * *****

Metacestode_Mizoram             CGTGTCCGTCTCTC--TCTCTTTCTGTGTGTGTGTGTGTGTGCGCGGGGG
T_taeniaeformis_Hyderabad       TGTGCGCGTATCTCGGTCTCGTGGTGTGTGTGTGTGCGTGGGAGCAGGGG
                                 ***  *** ****  **** *  ************ *** * ** ****

Metacestode_Mizoram             AGTGGGAGCCCGGTCCATCCCGGGGGCGGAGGGGTTTATACACATGCGCG
T_taeniaeformis_Hyderabad       ACCGCTCCACACGTCCATGCCGGAGGGGCAGAGACATGTGCACACGCGCG
                                *  *     *  ****** **** ** * ** *   * * **** *****

Metacestode_Mizoram             CGCGATTTTGTGTGTGTGTGTGCGTGTGTGCGAGGCGTAAGATAGTGATG
T_taeniaeformis_Hyderabad       CAC------GTGTTTGTGTGTGCGTGTGTGCGAGGCGTAAGATAGTGATG
                                * *      **** ************************************

Metacestode_Mizoram             GATGCGACGGTTGTGCAGTTGCGTCCGTCCGCGCCCCATCATGTGTCATG
T_taeniaeformis_Hyderabad       GATGCGACGGTTGTGCAGTTGCGTCCGTCCGCGCCCCATCATGTGTCATG
                                **************************************************

Metacestode_Mizoram             TGTTTGGTGTAGTGGTGATGGTTGTGCGTGAGTGTAAGGCGATGTGAAGT
T_taeniaeformis_Hyderabad       TGGTTGGTGTAGTGGTGATGGTTGTGCGTGAGTGTAAGGCGATGTGAAGT
                                ** ***********************************************

Metacestode_Mizoram             GGAGAAGTGGAATGGCATGGCAATAACTGTGCGCGGTGGATCACTCGGCT
T_taeniaeformis_Hyderabad       GGAGAAGTGGAATGGCATGGCAATAACTGTGCAC--TTAATCACTCGGCT
                                ******************************** *  *  ***********

Metacestode_Mizoram             CGTGTGTCGATGAAGGGTGCAGCCAACTGTGAGAATTAGTGTGTAGTAGC
T_taeniaeformis_Hyderabad       CGTGTGTCGATGAAGGGTGCAGCCAACTGTGTGA---------TAAAAGC
                                ******************************* **         **  ***

Metacestode_Mizoram             CAGAGGTGCTCTCGTA
T_taeniaeformis_Hyderabad       C---GGCCTACTTGT-
                                *   **    ** **
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Fig.2.3. Pairwise alignment – ITS1

Multiple alignment of COI of the query sequence with those of the three different geographical isolates showed the presence of 2.1% mismatches with no gap (Fig. 2.4).

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T_taeniaeformis_Japan      ATTCTTCCTGGATTTGGTATTATTAGACATATATGCTTAAGAATTAGTAT
T_taeniaeformis_Kazakhstan ATTCTTCCTGGATTTGGTATTATTAGACATATATGCTTAAGAATTAGTAT
Metacestode_Mizoram        ATTCTTCCTGGATTTGGTATTATTAGACATATATGTTTAAGAATTAGTAT
T_taeniaeformis_Kolkata    ATTCTTCCTGGATTTGGTATTATTAGACATATATGCTTAAGAATTAGTAT
                           ***********************************●**************

T_taeniaeformis_Japan      GTCTTCGGATGTGTTTGGTTTTTATGGTTTATTGTTTGCTATGTTTTCTA
T_taeniaeformis_Kazakhstan GTCTTCGGATGTGTTTGGTTTTTATGGTTTATTGTTTGCTATGTTTTCTA
Metacestode_Mizoram        GTCTTCGGATGTGTTTGGTTTTTATGGTTTATTGTTTGCTATGTTTTCTA
T_taeniaeformis_Kolkata    GTCTTCGGATGTGTTTGGTTTTTATGGTTTATTGTTTGCTATGTTTTCTA
                           **************************************************

T_taeniaeformis_Japan      TAGTTTGTTTAGGAAGAAGGGTGTGAGGTCATCATATGTTTACTGTTGGG
T_taeniaeformis_Kazakhstan TAGTTTGTTTAGGAAGAAGGGTGTGAGGTCATCATATGTTTACTGTTGGG
Metacestode_Mizoram        TAGTTTGTTTAGGAAGAAGGGTGTGAGGTCATCATATGTTTACTGTTGGG
T_taeniaeformis_Kolkata    TAGTTTGTTTAGGAAGAAGGGTGTGAGGTCATCATATGTTTACTGTTGGG
                           **************************************************

T_taeniaeformis_Japan      TTAGATGTAAAGACGGCTGTGTTTTTTAGTTCTATAACTATGATTATTGG
T_taeniaeformis_Kazakhstan TTAGATGTAAAGACTGCTGTGTTTTTTAGTTCTATAACTATGATTATTGG
Metacestode_Mizoram        TTAGATGTAAAGACGGCTGTGTTTTTTAGCTCTATAACTATGATTATTGG
T_taeniaeformis_Kolkata    TTAGATGTAAAGACGGCTGTGTTTTTTAGTTCTATAACTATGATTATTGG
                           **************●**************●********************

T_taeniaeformis_Japan      AGTTCCTACGGGTATAAAGGTTTTTACATGATTGTATATGTTGTTGAATG
T_taeniaeformis_Kazakhstan AGTTCCTACAGGTATAAAGGTTTTTACATGATTGTATATGTTGTTGAATG
Metacestode_Mizoram        AGTACCTACAGGTATAAAGGTTTTTACATGATTGTATATGTTGTTGAATG
T_taeniaeformis_Kolkata    AGTCCCTACAGGTATAAAGGTTTTTACATGATTGTATATGTTGTTGAATG
                           ***●*****●****************************************

T_taeniaeformis_Japan      CTCGAGTCAAAAAGAGTGATCCTGTTTTATGATGAATTGTTTCTTTTATT
T_taeniaeformis_Kazakhstan CTCGAGTCAAAAAGAGTGATCCTGTTTTATGATGAATTGTTTCTTTTATT
Metacestode_Mizoram        CTCGAGTCAAAAAGAGTGATCCTGTTTTATGATGAATTGTTTCTTTTATT
T_taeniaeformis_Kolkata    CTCGAGTTAAAAAGAGTGATCCTGTTTTATGATGAATTGTTTCTTTTATT
                           *******●******************************************

T_taeniaeformis_Japan      ATTCTGTTTACGTTTGGTGGGGTTACTGGTATAGTATTATCAGCTTGTGT
T_taeniaeformis_Kazakhstan ATTCTGTTTACGTTTGGTGGGGTTACTGGTATAGTATTGTCAGCTTGTGT
Metacestode_Mizoram        ATTCTGTTTACGTTTGGTGGGGTTACTGGTATAGTATTATCGGCTTGTGT
T_taeniaeformis_Kolkata    ATTCTGTTTACGTTTGGTGGGGTTACTGGTATAGTATTATCGGCTTGTGT
                           **************************************●**●********

T_taeniaeformis_Japan      TTTAGATAAAGTGTTACATGATAC
T_taeniaeformis_Kazakhstan TTTAGATAAAGTGTTACATGATAC
Metacestode_Mizoram        TTTAGATAAAGTGTTACATGATAC
T_taeniaeformis_Kolkata    TTTAGATAAAGTGTTACATGATAC
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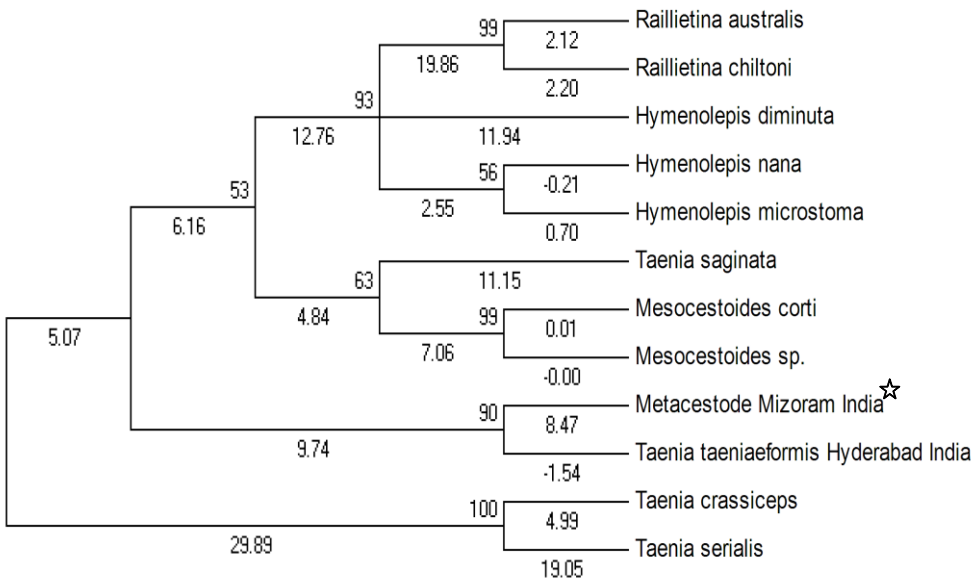
Fig.2.4. Multiple Sequence Alignment – CO1.

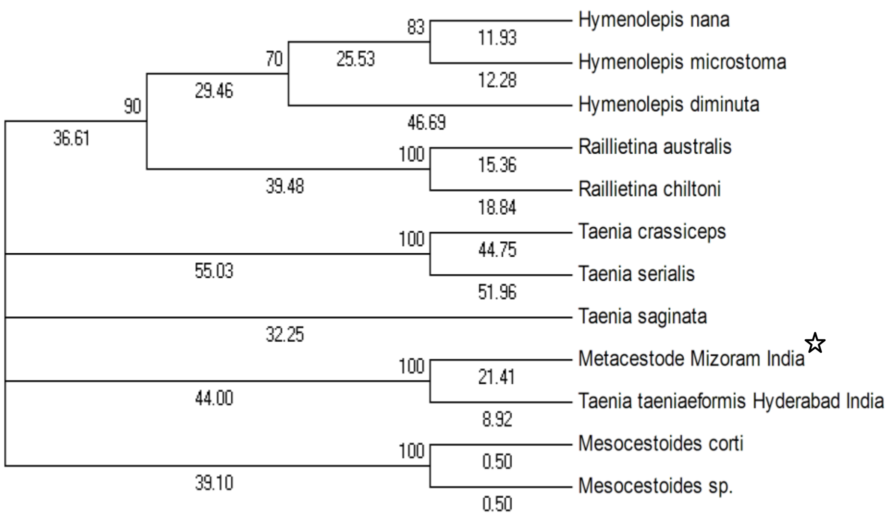
Construction of phylogenetic trees

Phylogenetic trees were obtained by comparing the ITS (1, 2) and mtCOI sequences of the metacestode with other cyclophyllidean cestode species using the NJ, UPGMA, ME and MP methods. However, since the topology obtained using all the character-based methods was found to be similar, herewith the results obtained through NJ along with MP method are depicted (Fig. 2.5- 2.7).

The topology of the trees obtained through both the methods for ITS2 emerged to be quiet similar placing both the *Taenia taeniaeformis* and the metacestode sequences in the same clade; the boostrap values of the nodes within the same species were large in all the regions 90% in NJ and 100% in MP trees.



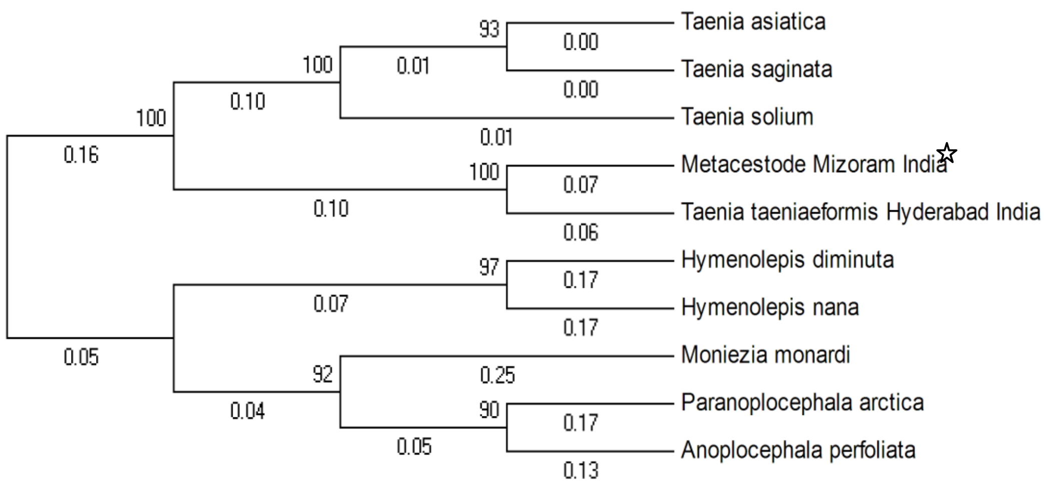
(a)



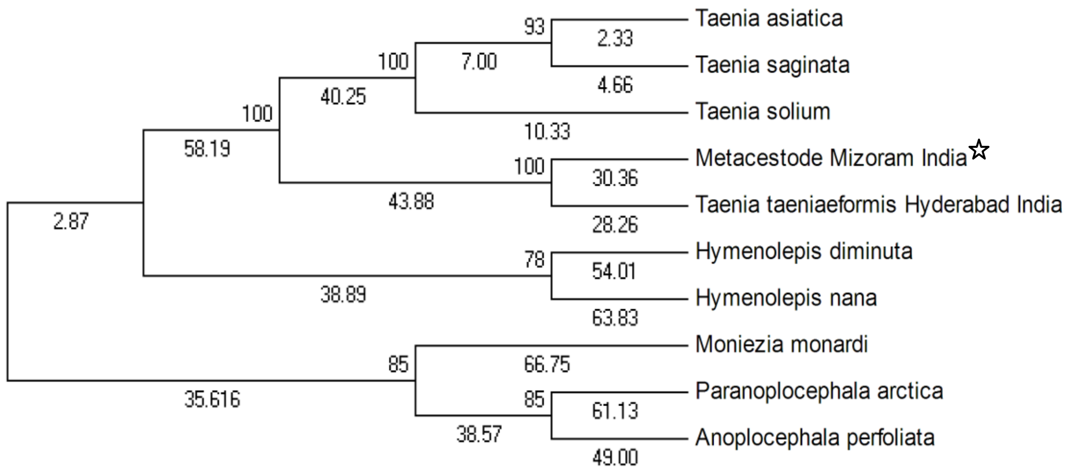
(b)

Fig.2.5. Phylogenetic trees of the metacestode ITS2- (a) NJ, (b) MP. (☆Query sequence)

For ITS1 also the topology of the trees obtained was found to be similar placing the *Teania taeniaeformis* and the metacestode sequences in the same clade with high bootstrap value of 100% in both NJ and MP trees (Fig. 2.6).



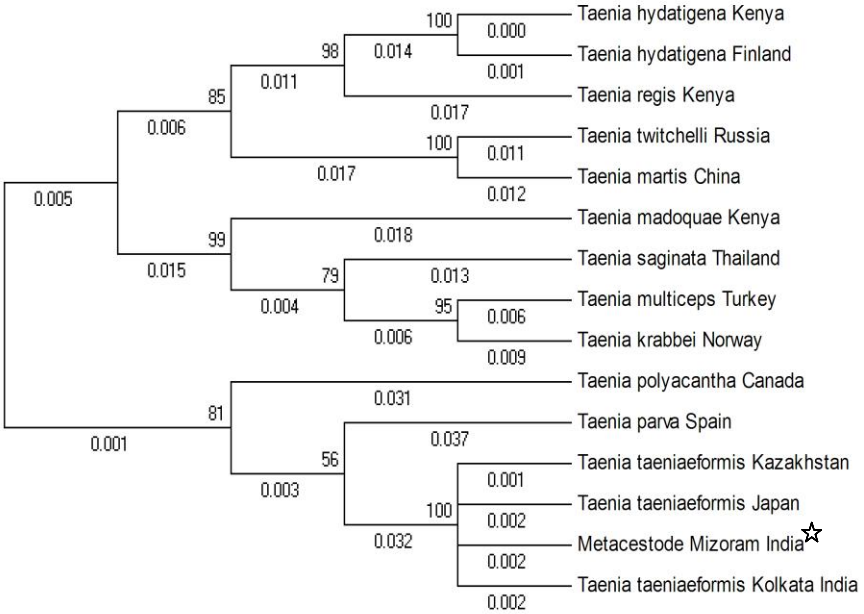
(a)



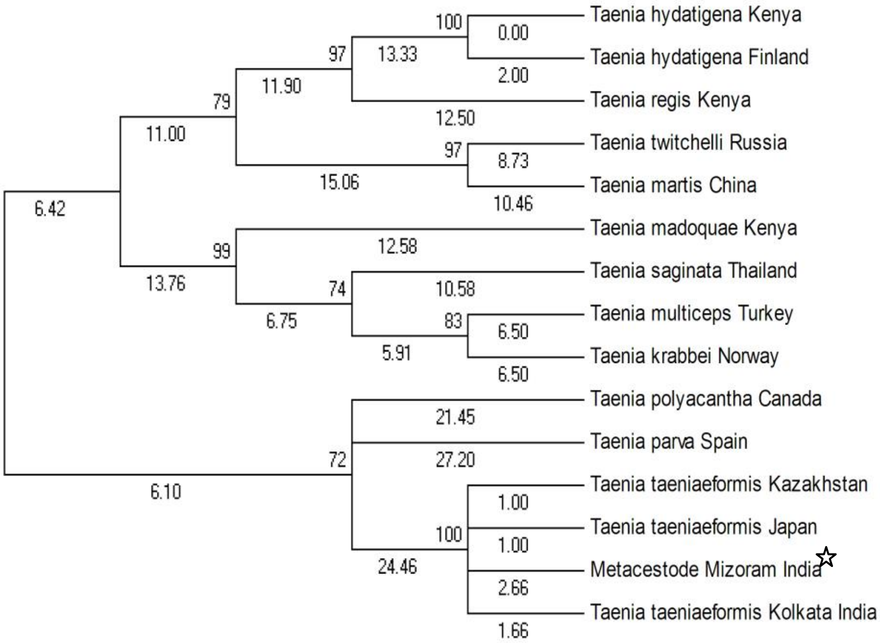
(b)

Fig.2.6. (a) NJ and (b) MP trees of the metacestode ITS1, showing bootstrap values and distance (☆Query sequence)

Similarly, in the trees constructed for COI, the query sequences are placed in the same clade with *T. taeniaeformis* isolates from Hokkaido (Japan), Kazakhstan and Kolkata (India), showing high bootstrap values of 100%.



(a)



(b)

Fig. 2.7. (a) NJ and (b) MP trees of the metacestode COI, showing bootstrap values and distance (☆Query sequences)

The topology of the trees obtained through both the methods emerged to be quiet similar placing both the *Taenia taeniaeformis* and the query sequences in the same clade; the boostrap values of (90% and above) of the nodes within the same species were large in all the three regions.

Hymenolepis diminuta: PCR amplification of ITS2 region and its analysis

PCR amplification of ITS2 region of the adult cestode from the intestinal lumen and identified as *Hymenolepis diminuta* on the basis of morphological criteria, showed a single band of size 455bp (Fig. 2.8.).

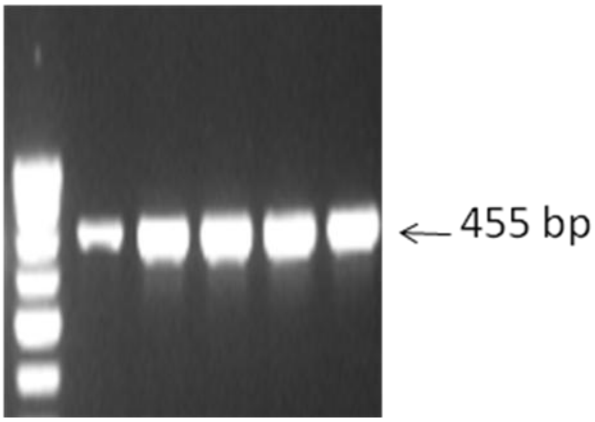


Fig. 2.8. PCR product of the *H. diminuta* using primer set 3S-A28 for ITS2

The nucleotide sequences obtained for ITS2 are shown below as raw data:

GATCACGAATCGATGATGAGTGCAGCCAACTGCTGTGATACTCTCATGTGAATCGCAGAC
TGCTTTGAACATCGATACCTTGAACGCATATTGCGGCCATAGGCTTGCCTATGGCCACGTC
TGTCTGAGCGTCGGCTTATAAACTATCACTGCGCGTAATAAGCAGTGGATTGGGAGAGTG
CCGTGATTGCAGTAGTTATGTGTGTGTATATGTGTGTTCCCCGAGTGGGAACAAAAGGGG
AGCCGGTTCTTCTCTTGCAGGGATTATCACAGCCATTGCCATTGAAAACGCTGATGCCCGC
GCCGCCCGAGATAAAAAATAAATAAACCAGGCACCTGTTATCCCCCCACCCCTGTTTTTAC
TTTGACACATGCTCCCCCACGGGGGGCACAGTGTGCACGAAAAATAGGTGGGGGGGGGA
AAAAATTTGGGTTACTCAAACTC

The sequences obtained from the PCR products were put to BLAST and compared with other available cyclophyllidean cestode sequences from GenBank (Table 2.2)

Table 2.2. ITS2 sequences of *Hymenolepis diminuta* and its various geographical isolates and other cyclophyllidean cestodes used in the study with respective GenBank accession numbers

Name of parasites	Host	Geographical Isolate	Accession No.
*Hymenolepis diminuta**	*Rattus rattus*	Mizoram, India	FJ 939132
Hymenolepis diminuta	*Rattus norvegicus*	Japan	AB494475
Hymenolepis diminuta	Laboratory rodents	USA	AB494474
Hymenolepis diminuta	Laboratory rodents	Australia	AF461125
Hymenolepis microstoma	Laboratory rodents	Japan	AB494478
Hymenolepis nana	*Mus musculus*	Japan	AB494476
Anoplocephala perfoliata	*Equus caballus*	Germany	AJ578153
Taenia taeniaeformis	*Rattus rattus*	Mizoram, India	FJ939133
Lineolepis scutigera	Holarctic shrew	Lithuania	GU299858

(Query sequence*)

In multiple sequence alignment of the query sequence with the sequences of *H. diminuta* Japan isolate showed maximum similarity, though with 8.1% mismatches (Fig 2.9)

```
CLUSTAL W (1.81) multiple sequence alignment


H_diminuta_Japan          ATCGATGAAGA-TGCAGCCAACTG-TGTGA-ATTAATGTG-AATCGCAGACTGCA-CGCA
H_diminuta_USA            ATCGATGAAGA-TGCAGCCAACTG-TGTGA-ATTAATGTG-AATCGCAGACTGCA-CGCA
H_diminuta_Australia      ATCGATGAAGAGTGCAGCCAACTG-TGTGA-ATTAATGTG-AATCGCAGACTGGAACGCA
H_diminuta_Mizoram        ATCGATGATGAGTGCAGCCAACTGCTGTGATACTCTCATGTGATCGATACCTTGAACGCA
                          ******** ** ************ ***** * *    **  ****    **  * ****

H_diminuta_Japan          TATTGCGGCCATAGGCTTGCCTATGGCCACGTCTGTCTGAGCGTCGGCTTATAAACTATC
H_diminuta_USA            TATTGCGGCCATAGGCTTGCCTATGGCCACGTCTGTCTGAGCGTCGGCTTATAAACTATC
H_diminuta_Australia      TATTGCGGCCATAGGCTTGCCTATGGCCACGTCTGTCTGAGCGTCGGCTTATAAACTATC
H_diminuta_Mizoram        TATTGCGGCCATAGGCTTGCCTATGGCCACGTCTGTCTGAGCGTCGGCTTATAAACTATC
                          ************************************************************

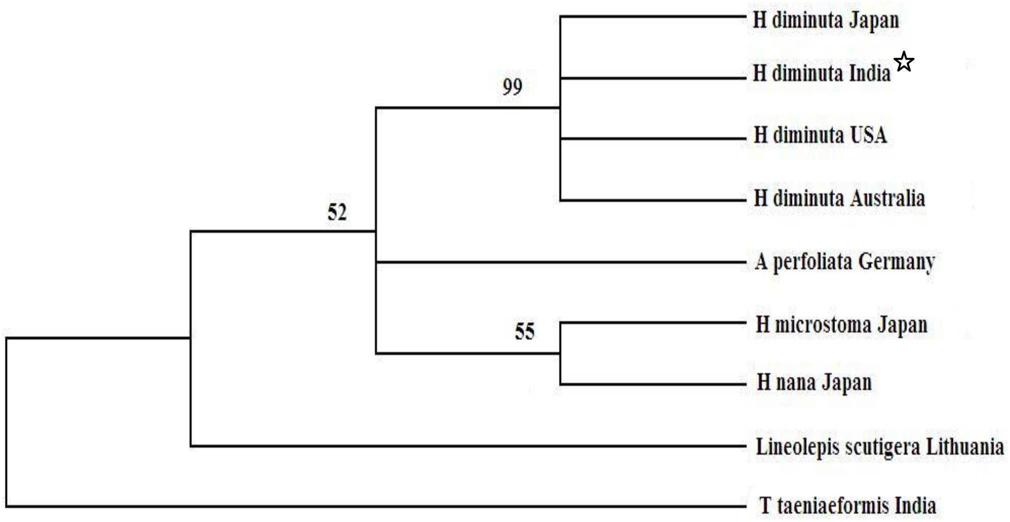
H_diminuta_Japan          ACTGCGCGTAATAAGCAGTGGCTTGGGAGAGTGCCGTGATTGCAGTAGTTATGTGTGTGT
H_diminuta_USA            ACTGCGCGTAATAAGCAGTGGCTTGGGAGAGTGCCGTGATTGCAGTAGTTATGTGTGTGT
H_diminuta_Australia      ACTGCGCGTAATAAGCAGTGGCTTGGGAGAGTGCCGTGATTGCAGTAGTTATGWGTGTGC
H_diminuta_Mizoram        ACTGCGCGTAATAAGCAGTGGATTGGGAGAGTGCCGTGATTGCAGTAGTTATGTGTGTGT
                          ********************* ******************************* *****

H_diminuta_Japan          --ATATGTGTGTTGCGTGCGCGCTACTACTGCTGCAGTTGGGGCTTCTCTTTAAGGTATT
H_diminuta_USA            GTATATGTGTGTTGCGTGCGCGCTACTACTGCTGCAGTTGGGGCTTCTCTTTAAGGTATT
H_diminuta_Australia      GTATATGTGTGTTGCGTGCGCGCTACTACTGCTGCAGTTGGGGCTTCTCTTTAAGGTATT
H_diminuta_Mizoram        --ATATGTGTGTTCCCCGAGTGGGAACAAAAGGGGAGCCGGTTCTTCTCTTGCAGGGATT
                            *********** *  * * *  *  *     * **  **  ********  *** ***

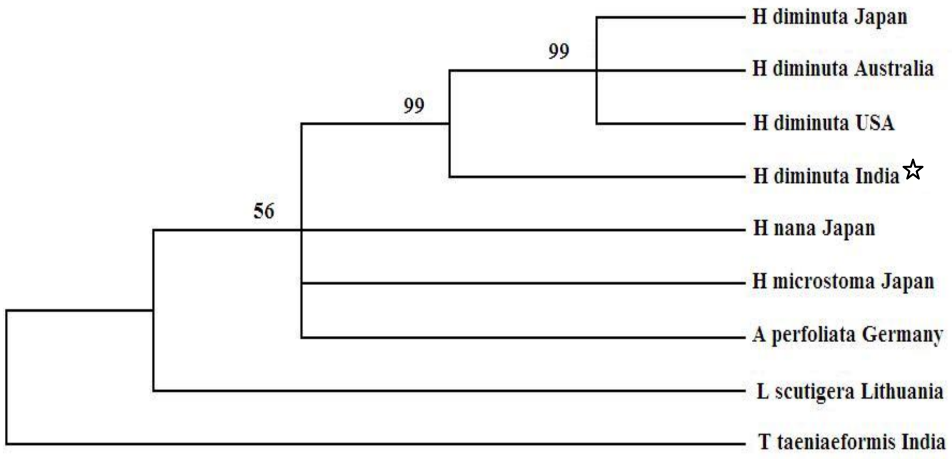
H_diminuta_Japan          ATCACAGCCATTGCCATTGCGAATGGTGATGC
H_diminuta_USA            ATCACAGCCATTGCCATTGCGAATGGTGATGC
H_diminuta_Australia      ATCACAGCCATTGCCATTGCGAATGGTGATGC
H_diminuta_Mizoram        ATCACAGCCATTGCCATTGAAAACGCTGATGC
                          *******************  ** * ******
```

Fig.2.9. Multiple sequence alignment – *H. diminuta* ITS2

The topology of the trees obtained for ITS2 turned out to be quiet similar placing the *H. diminuta* isolates from Japan, India, USA and Australia in the same clade, with high bootstsrap value of 99% for both NJ and MP trees (Fig. 2.10)



(a)



(b)

Fig. 2.10. (a) NJ and (b) MP trees of *H. diminuta* ITS2 showing bootstrap values (☆Query sequences)

ITS2 Secondary structure analysis and GC content

ITS2 Secondary Structure

In ITS2 of *T. taeniaeformis* and *H. diminuta* distinct hallmark of a core secondary structure was seen; (i) four helices (ii) helix III being the longest, (iii) an UGGU motif in the 5' end (Deviations like UGGGU, UGG or GGU) are also seen and U-U mismatch in the second helix which contributed to the overall consensus structure highly indicative for a conserved common core ITS2 secondary structure (Fig. 2.11-2.12).

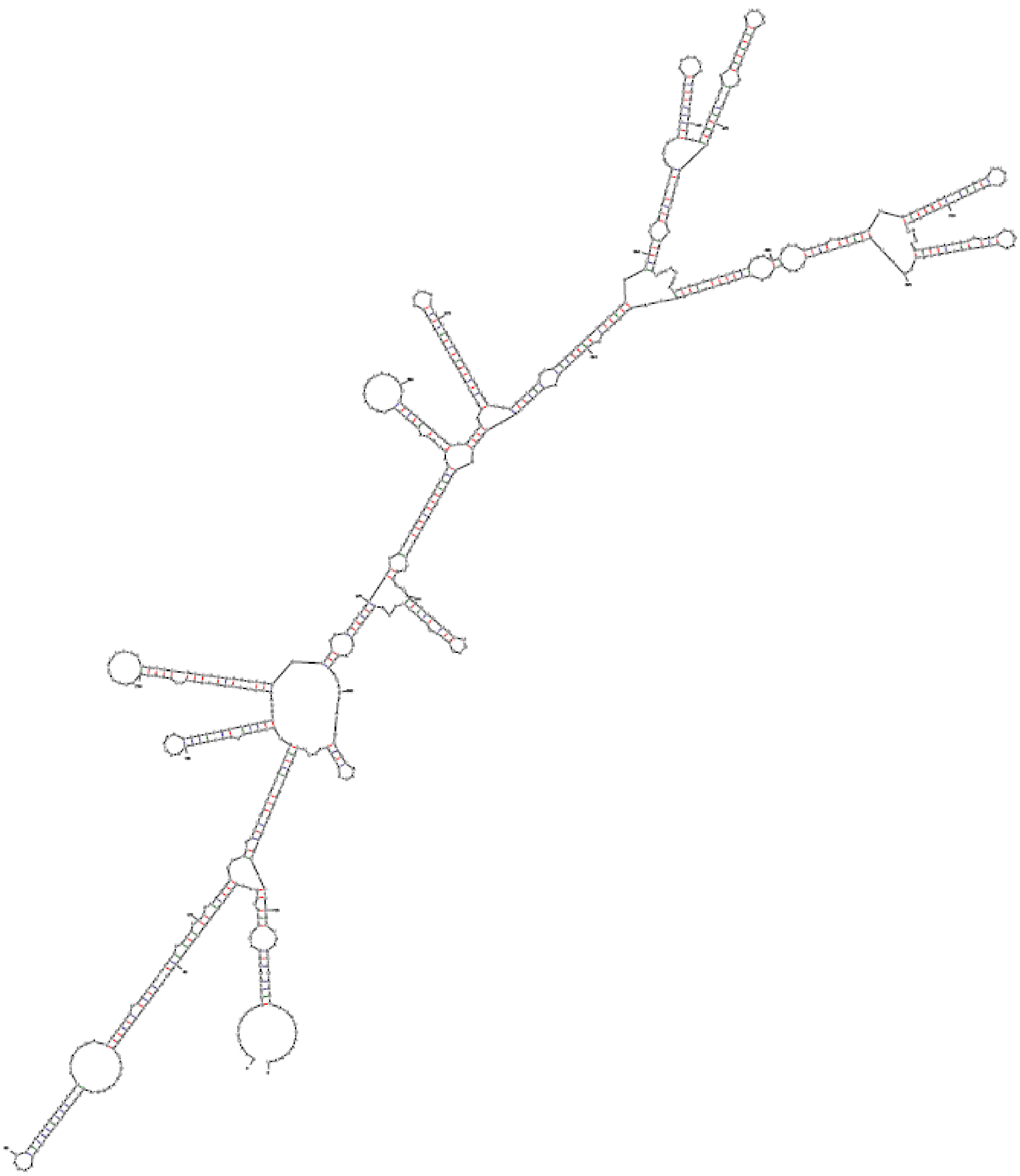


Fig.2.11. ITS2 Secondary structure of metacestode (= *Taenia taeniaeformis*) (GenBank Accession No. FJ939133); dG=-279.49 [initially -301.30]

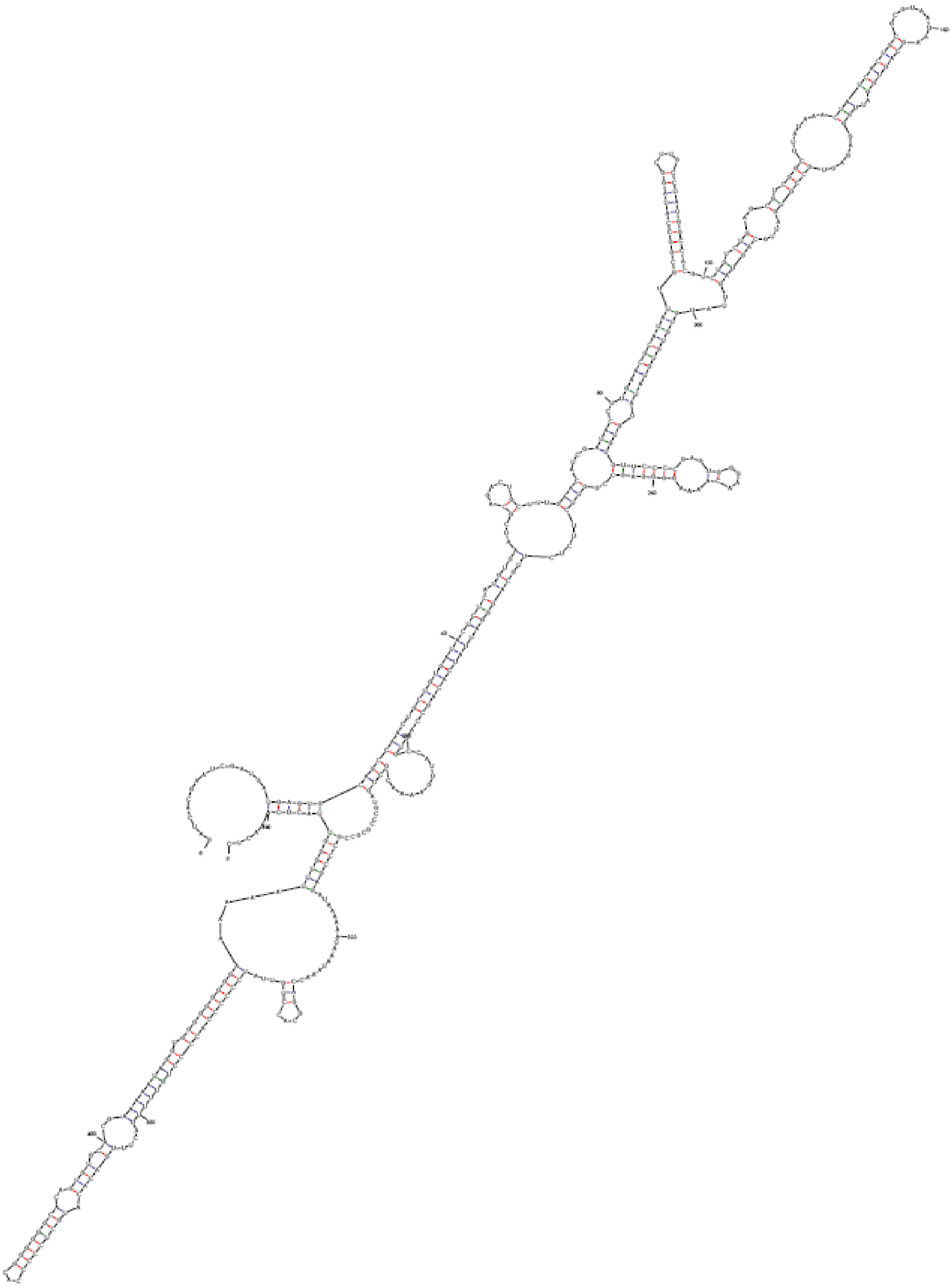


Fig.2.12. ITS2 Secondary structure of *H. diminuta* (GenBank Accession No. FJ939132); dG=147.22 [initially -160.30]

GC Content:

The GC content in the ITS2 region was calculated using oligo calculator, with regard to the metacestode (*Taenia taeniaeformis* Accession no. FJ 939133.1) the GC content was found to be 61.6%, whereas for *H. diminuta* (FJ939132.1) it was as low as 43.7%. Besides the two species, the GC content was also calculated for ITS2 region of several other cyclophyllidean cestode species, as depicted in Table 2.3. The GC content varied from 54.6% to 62.6% for *Taenia* spp. and for species of *Hymenolepis* it ranged from 43.7% to 54%. Three *Taenia* species showed a higher GC content compared to others.

Table.2.3. Percentage of GC content in the ITS2 region of various cyclophyllidean cestodes

Organism name	GenBank Accession No.	GC Content
Taenia saginata	AY825540.1	62.4%
Taenia serialis	DQ099571.1	62.6%
Taenia crassiceps	DQ099564.1	57%
Taenia taeniaeformis	FJ939133.1	61.6%
Hymenolepis nana	AB494477.1	50.5%
Hymenolepis diminuta	FJ939132.1	43.7%
Raillietina beveridgei	AY382318.1	50.5%
Mesocestoides spp.	AF119697.1	58.8%
Anoplocephala perfoliata	AJ578153.1	46%
Echinococcus granulosus	AY389985.1	59%

DISCUSSION

The observations on the metacestode morphology clearly showed the presence of double rows of taeniid hooks and four lateral suckers, which are the distinguishing characters for *Taenia* spp (Al-Jashamy and Islam, 2007). The size and the number of hooks are important features for identification of Taeniidae species. The numbers of hooks reported in the metacestode under study were also found to be in confirmity with those of *Taenia taeniaeformis* (Verster, 1969; Al-Jashamy and Islam, 2007).

The rDNA cluster has been widely used for taxonomic studies as it is highly repeated and contains highly variable regions flanked by conserved regions (Hillis and Dixon, 1991). Mitochondrial genes such as cytochrome *c* oxidase sub units COI, II have been used to study phylogenetic relationships among related organism as they have fast evolutionary rate (Lecanidou et al., 1994). In analysis of the sequences of the rDNA ITS and the mtCOI and comparing with the so far known sequences of other cyclophyllidean cestodes, the query (metacestode and the adult worm) sequences showed close similarity with the sequences of *T. taeniaeformis* for metacestode and *H. diminuta* for the adult worm, with high bootstrap value of 90% and above. As a general rule in phylogenentic analysis if the bootstrap value for a given interior branch of a phylogenetic tree is 70% or higher, then the topology at that branch is considered reliable or correct (Hillis and Bull, 1993). The high bootstrap values of the query sequence with *T. taeniaeformis* indicates perfect phylogenetic accuracy and reliable grouping. In the present study when COI region of the metacestode sequences were aligned with three different isolate only a few mismatches were seen without any gaps. But alignment of the ITS regions of the Query sequence shows the presence of large number of mismatches for both *T. taeniaeformis* and *H. diminuta*. Several criteria such as morphology, infectivity, and protein component and restriction fragment length polymorphism of the parasite DNA have also been used in describing intraspecific variations among the different isolates of *T. taeniaeformis* (Azuma et al., 1995). Intraspecific variation in *T. Taeniaeformis* has also been reported taking infectivity to intermediate host as the sole criterion (Brandt and Sewell, 1981; Conchedda and Ferretti, 1983). Moore and Brooks (1987) constructed a phylogenetic tree based on the biological and morphological characters of the adult taeniid parasites and concluded that the phylogenetic relationship of the family Taeniidae is incongruent with both definitive and intermediate host phylogenies.

Intra-specific ITS diversity has been previously reported for the cyclophyllidean cestode *Taenia solium* (Hancock et al., 2001) and the diphyllobothriidean *Ligula intestinalis* (Bouzid et al., 2008), the greatest variation in *T. solium* sequence variants was accounted for by different copies of repeat elements, especially the GGTC repeat, that varies from five to 10 copies and the sequence variation of the ITS1 within the individual tapeworms was as high as the variation between *T. solium* specimen from different geographic regions and difference in the intra-genomic ITS2 sequence structure was also found in the diphyllobothriidean *L.*

intestinalis. Bouzid et al., 2008 observed two type of intra-genomic variability. First are sequences which are characterized by minor changes in sequence structure and clustered within a single mitochondrial cox1 clade, whereas second type was represented by highly divergent ITS variants that clustered in several different lineages. For some Trematodes and Cestodes asexual multiplication of their larval stages is associated with substantial DNA replication, which may lead to sequence heterogeneity in ITS (van Hewerden et al., 2000). The presence of large amount of mismatches in pairwise alignment of the ITS regions in both metacestode of *Taenia* sp. and *H. diminuta* may also be due to the presence of intra-specific ITS diversity within the cestode which has been previously reported for the cyclophyllidean cestode by Hancock et al. (2001).

The ITS2 region is a requisite in ribosome biogenesis (Cote and Peculis, 2001) and its gradual removal from mature rRNA is driven by its specific secondary structure. In the present study the secondary structure of ITS2 of *H. diminuta* shows the presence of four helices which are the characteristics features of the ITS2 secondary structure whereas in case of *T. taeniaeformis* differences in the secondary structure were found within the same genus.

On the basis of the morphological similarities, supplemented by close matching of the ITS and mtCOI sequences of the metacestode under study with *T. taeniaeformis* and the adult cestode from the intestinal lumen with *H. diminuta* it can be concluded that the parasite recovered from the liver cyst of rodents in the study area indeed represents *Cysticercus fasciolaris*, i.e, the metacestode of *T. taeniaeformis,* the adult of which occurs in the various carnivorous animals and the adult cestode found in the intestinal lumen is *Hymenolepis diminuta* which commonly infects the rodents host.

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Chapter – 3

Hepatic Histopathological studies in metacestode and *Capillaria*- infected rodents

INTRODUCTION

Importance of wild rats as reservoir of helminth infection has long been known (Firlotte, 1948). Researchers have used rodent species infected with these parasites as potential animal models for the studies of hepatic fibrosis and cirrhosis and parasitic carcinogenesis. However, there is scanty information and little scientific literature related to rat-borne liver infection.

Both *Capilalria hepatica* and *Taenia taeniaeformis* have been reported as a common parasite of rodents worldwide. Frequent occurrence of *C. hepatica* has been reported from Asia; the prevalence of infection ranges from as low as < 20% to as high as 100% (Seong et al., 1995; Namue and Wongsawad, 1997; Claveria et al., 2005; Tung et al., 2009). As high as 80% prevalence was reported from the city of Baltimore, Maryland, USA (Farhang-Azad, 1977; Conlogue et al., 1979; Easterbrook et al., 2007). In Africa a low prevalence of infection was reported from Norway rats in Egypt (El- Nassery et al., 1991) and in black rats in Ethiopia (Farhang-Azad and Schlitter, 1978). With regard to Europe, a low prevalence (> 2.9%) of *C. hepatica* infection was reported from Norway rats in Croatia (Stojcevic et al., 2002) and considerably higher prevalence (41-44%) in England (Webster and MacDonald, 1995), France (Davoust et al., 1997) and Italy (Ceruti et al., 2001).

T. taeniaeformis has been reported from the black rats in Asia (Seo et al., 1964; Seo et al., 1968; Seong et al., 1995; Claveria et al., 2005; Singla et al., 2008), in Norway rats in Canada (Firlotte, 1948), the USA (Andrews and White, 1936) and Europe (Webster and MacDonald, 1995; Battersby et al., 2002) and in both black and Norway rats in New Zealand (McKenna, 1997).

For both the parasites wild common rats act as reservoir of infection and through predation help to spread the infections. Till date 38 cases of human infection with *C. hepatica* have been recorded (Nabi et al., 2007) and children under 5 years of age are at particular risk of developing the disease (Berger et al., 1990; Spratt and Singleton 2001); human infections with *T. taeniaeformis* are rather rare and only a few cases have been reported (Hoberg, 2002).

T. taeniaeformis is a taeniid cestode found in the intestine of cats and other felines and carnivores as definite hosts. Rodents serve as the intermediate host, in which the larval form or the metacestode, *Cysticercus fasciolaris,* develops in the liver and other organs as a fluid-filled bladder worm inducing fibroplasia and progressive inflammation that may eventually progresss to form fibrosarcoma (Hanes 1995). Sarcomas of the rat liver due to the presence of Cysticercii have long been known; several experiments using the bladder worm to induce malignant growth of the connective tissues in the liver of rats were successfully conducted as early as the first quarter of the twentieth century (Bullock and Curtis 1920, 1924, 1926, 1928). Liver fibrosarcoma due to the presence of *C. fasciolaris* was suggested to be an apropriate model for studying parasitic carcinogenesis and pathogenesis in wild rats (Tucek et al., 1973).

C. hepatica is a zoonotic nematode found in the liver of rodents and other lagomorphs that can also parasitize man (Cochrane et al., 1957; Cislaghi & Radice, 1970; Berger et al., 1990; Choe et al., 1993) and which has been the most frequently encountered species in wild and house rodents (Junker et al., 1998; Seong et al., 1998). A high prevalence of this parasite in wild rats was observed in northern part of India (Mittal 1980; Gupta and Trivedi 1988; Somvanshi et al., 1995; Chahota et al., 1997). *C. hepatica* has a direct life cycle which takes place in the liver of a single host (Calle, 1961); both male and female worms are found within the liver parenchyma where they live and unembryonated eggs are released only after the host dies and its liver decays or when the host is eaten by a predator carnivore. Under favourable conditions the ova embryonate and become infective, the larvae hatch in the intestine and migrate via the portal vein into the liver where they become mature and mate. The female worms of *C. hepatica* die soon after laying eggs and disintegrate inside the liver freeing thousands of eggs in the liver, forming focal necro-inflammmatory lesion that heals by encapsulation, calcification and resorption (Luttermoser, 1938; Ferreira and Andrade, 1993; Gotardo et al., 2000). Several studies have been carried out on the pathogenesis of *C.hepatica* and its effect on the liver tissue. In laboratory mice, the infection can reduce the reproductive output or even cause death of the host (Luttermoser 1938; Singleton and Spratt 1986).

In view of the frequent occurrence of the two parasites in the rodents collected during the bamboo flowering period in Mizoram, the present studies aimed to find out the effect of the metacestode and *C. hepatica* on the liver parenchyma of the hosts and to ascertain their potential as a tool for biological control of rodents. For the purpose a histopathological approach was adopted.

MATERIALS AND METHOD

As mentioned in the preceding chapters, a total of 280 rodents belonging to 9 species representing 6 genera that are commonly prevalent rodent species in the region were collected from 12 different locations.

Upon necropsy of the host animals, the hepatic parenchyma was observed to be studded with cream-coloured soft cysts, which upon opening were found to contain viable creamy white metacestode, i.e., *Cysticercus fasciolaris*. Presence of *C. hepatica* was also observed by gross examination of the liver tissue and the nematode was easily recognized externally by the presence of irregular white or yellowish white nodules containing the eggs or adult worms and scattered all over the surface of the liver.

The liver tissue containing both the parasites was separated and washed with PBS and stored at -40℃ for frozen sections. The fresh frozen tissues of the infected and control (uninfected) liver were sectioned in cryostat (Model No LEICA CM 1850) at 14μm thickness at -20℃, and stained. For histopathological studies, alteration if any in collagen, lipids and eosinophils were used as the parameters, for detection of which Masson trichrome, Hematoxylin and eosin (H&E) and Sudan black respectively were used following Pearse (1968).

RESULTS

In 22.3% of the rodent hosts creamy white-coloured cysts were observed mostly in the caudal and lateral lobes of the liver (Fig. 3.1). The number of cysts collected from a single host varied from 1-15. Morphological examination of the stained parasites revealed typical characteristics of taeniid cestodes with the presence of an armed rostellum having two rows of large and small hooks and four prominent suckers on the scolex, a long neck and pseudo segmentation of the entire body length with a terminal bladder, which is in consistence with the larvae of *T. taeniaeformis*.

In 24.4% of the hosts examined, granulomatous lesions associated with eggs or portion of damaged adult nematode worms, consistent with *Capillaria hepatica,* were detected either scattered on the liver surface or localized in a single lobe. Prevalence of the metacestode was found to be highest in the rodent species *R. norvegicu*s, whereas *R. nitidus* showed highest prevalence of *C. hepatica*.

Histopathological studies of the infected liver revealed distortion of the normal morphology of the liver parenchyma and inflammation due to the presence of both the parasites (Fig. 3.3). The presence of metacestode was revealed inside a well defined fibrous tissue capsule. The cells appeared spindle shaped and clustered together with abnormal nuclei in and around the area where the metacestode occurs; in some area the cells seemed to be larger as compared to normal and a large numbers of them were multinucleated and the normal architecture of the liver cells seems to be altered (Fig. 3.3A). As revealed with Masson's trichrome stain, numerous neoplastic cells were observed with an abundant deposition of blue coloured collagen sheath (Fig. 3.3B).

In the *C. hepatica* infected liver; the lipid content was found to be more than the uninfected tissue, more so on the surface of the *C. hepatica* eggs as compared to the hepatic parenchyma (Fig. 3.3C). Partially calcified worm debris and collections of immature and mature eggs were found in the area where *C. hepatica* worms occurred and disintegrate. Granulomatous lesions surrounding the eggs of *C. hepatica* were detected (Fig. 3.3D); sometimes the lesion contained only a calcified core. Besides these inflammatory lesions there also occured septal formations within the infected liver (Fig. 3.3E). The clusters of *C. hepatica* eggs on the liver parenchyma were clearly visible as having an ovoid structure with bipolar caps. Abundant eosinophilic cytoplasm was observed in the region where the metacestode of *Taenia* and *C. hepatica* occurred red adjacently (Fig. 3.3F).

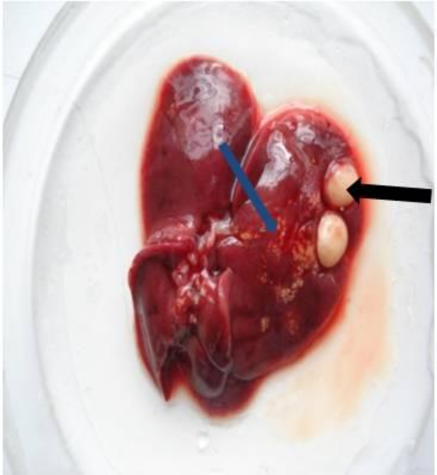

Fig. 3.1. *Taenia* sp. cysts attached to the liver lobe (black arrow) and irregular white streak (blue arrow) showing the presence of *C. hepatica*

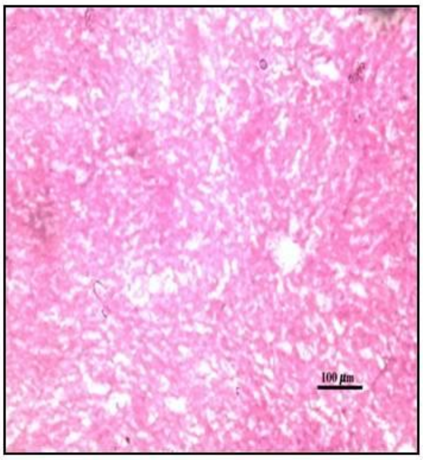

Fig. 3.2. Uninfected liver (Haematoxylin & Eosin)

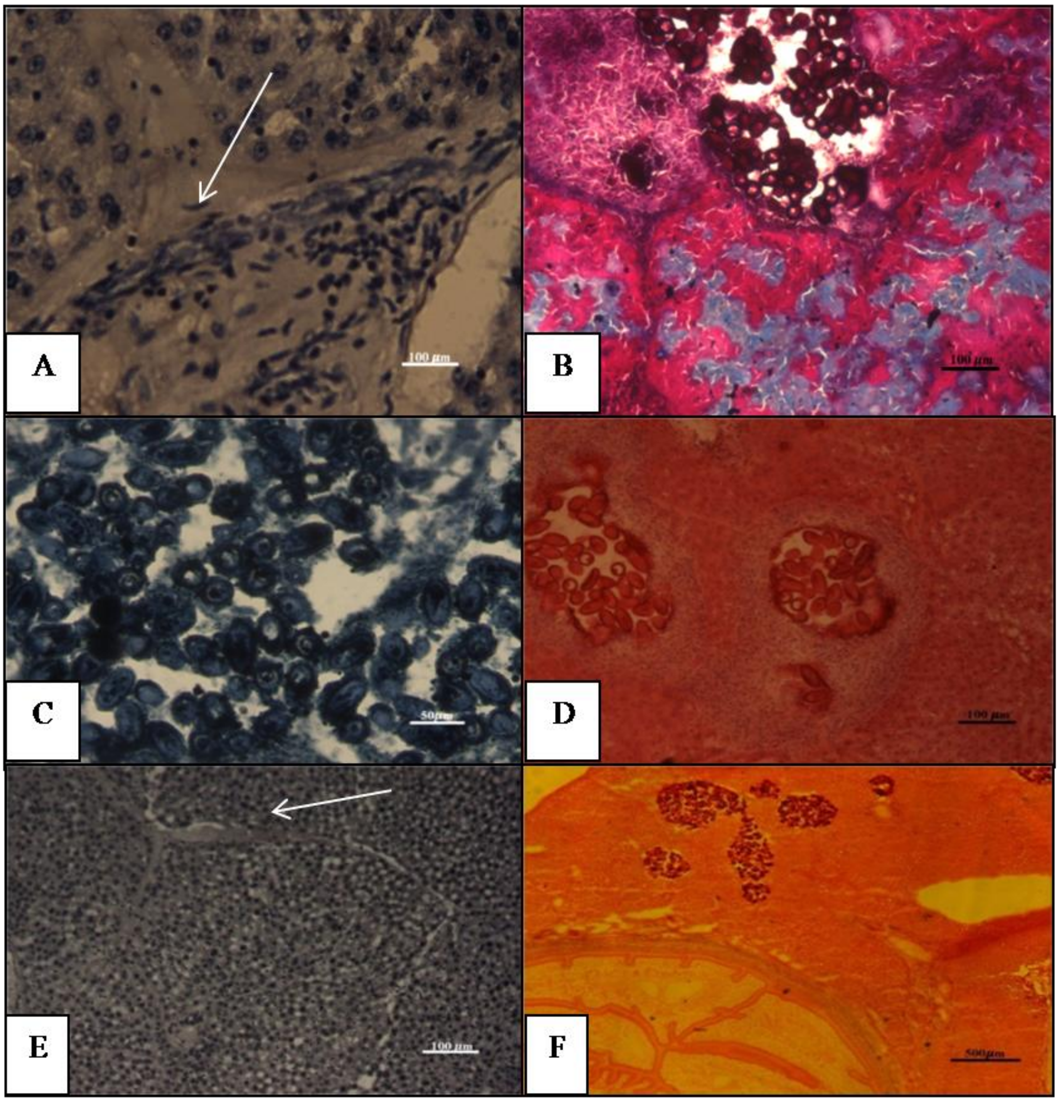

Fig. 3.3 A. Spindle shaped cells aggregating near the area where the parasite occurs (haematoxylin).

B. Infected liver tissue, showing nuclei (black), Cytoplasm (red) and large deposition of collagen sheath (blue)- Masson's trichrome stain.

C. Presence of lipids in the liver tissue infected with *C. hepatica*- Sudan black.

D. Granulomatous liver lesion with clusters of *C.hepatica* eggs (H&E).

E. A thin fibrous septa appears across the liver parenchyma (Haematoxylin)

F. Haematoxylin and eosin stain shows fibrous tissue encapsulation of the metacestode and abundant eosinophilic cytoplasm around the area where both the parasites occurs adjacently (H&E).

DISCUSSION

The presence of the metacestode of *Taenia* and *C.hepatica* altered the normal morphology of the liver of rats. Hepatic sarcoma associated with *Teania* sp. in wild rats has been reported (Tucek et al., 1973). A granulomatous reaction in the liver is a common histopathological finding in naturally infected rats (Davous et al., 1997; Ceruti et al., 2001). These focal inflammatory lesions are determined by the presence of live, dying or dead worms and their eggs. In consistence with the present findings spindle-shaped pleomorphic neoplastic invading liver parenchyma and the cells separated by collagen have been reported earlier in Sprague Dawley rats (Hanes, 1995; Wohrmann and Teredesai, 2002); in addition infiltration of eosinophils, plasma cells and macrophages was also reported (Kumar et al., 2006). Lethargy, weigh loss, anoerexia and sudden death are the clinical signs that have been associated with the presence of the meatcestode in the liver of rats (Tucek et al., 1973; Hanes, 1995). In case of *C. hepatica* infection, focal encapsulating fibrous response caused by the dead worms eventually progresses to form septal fibrosis and leads to cirrhosis (de Souza et al., 2000). Septal fibrosis is a frequent morphological type of hepatic fibrosis, which is represented by thin and straight fibrous septa that dissect the liver parenchyma. Its pathogenesis is a matter of considerable interest (Bhunchet and Wake, 1992; Bhunchet and Fujeida, 1993; Onori et al., 2000). Experimental data obtained after *C. hepatica* egg infection have shown the presence of focal lesions, which are necessary for the development of septal fibrosis (Santos et al., 2001). Although focal lesions and septal fibrosis run on independent pathways, the induction of the latter is triggered during early infection (Gomes et al., 2006). Consistent with the present findings, Oliveira and Andrade (2001) reported the occurrence calcified adult worms and immature eggs within the focal lesion delimited by fibrous capsule and also the formation of mild septal fibrosis. Recently, Yi et al. (2010) reported the presence of both *C. hepatica* and *T. taeniaeformis* in 31.5% of the wild rats captured in South Korea and also observed that the lesion caused by the parasite were closely associated with pulmonary arteriolar hypertrophy, which may represent the effects of pulmonary hypertension. These authors suggested that *C. hepatica* infected rats might be useful as an animal model for the studies of portopulmonary hypertension. Kataranovski et al. (2010) also reported the presence and characteristics of *C. hepatica* and *T.*

taeniaeformis liver infection in wild *Rattus norvegicus* from Belgrade, Serbia; fibrous inflammatory and granulomatous reactions surrounding the *C. hepatica* eggs were reported, an observation which is corroborated by the present findings as well. Considering the effects of both the parasites on the liver of the rodent hosts it would be worthwhile to investigate further and ascertain the possibility of using these parasites as a potential biological control tool.

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Summary

The present studies aimed at exploring and typifying the helminth parasites diversity in rodent population in Mizoram, to study the distribution pattern, species composition of the parasite groups prevalent in the rodent hosts and morphological, ultrastructural and molecular characterization of the parasites species and to identify if any of the helminth species emerges as plausible infectious agents that could serve as a potential tool for biological control of the rodent pests. The study has been divided into three main chapters as follows.

I. The parasite spectrum:

- 280 rodents, all of which belong to the family Muridae and are represented by six genera and nine species (namely *Rattus rattus*, *R. nitidus*, *R.norvegicus*, *Bandicota bengalensis*, *Berylmys mackenziei*, *B.bowersi*, *Mus musculus*, *Niviventer fulvescens* and *Cannomys badius*) were collected from 12 different localities in Mizoram.

- The survey results revealed that in the parasites spectrum nematode emerged as the predominant group represented by 9 genera and species- *Trichuris muris*, *Capillaria hepatica*, *Trichosomoides crassicauda*, *Heterakis spumosa*, *Rictularia* sp., *Syphacia obvelata*, *Aspiculuris* (*Paraspiculuris*) *pakistanica*, *Nippostrongylus brasiliensis* and *Hepatojarakus bandicoti*.

- Cestodes of the cyclophyllidean group comprised 4 genera represented by *Hymenolepis diminuta*, *Rodentolepis* sp., *Raillietina celebensis* and the metacestode *Cysticercus fasciolaris* with *Hymenolepis diminuta* being the most encountered species.

- *Moniliformis moniliformis* was the lone acanthocephalan recovered.

- Infection by any trematode was found to be conspicuously missing.

- Among all the host species, *R. rattus* harboured the widest range of helminth parasites with the presence of 8 nematode and 4 cestode species followed by *R. nitidus,* in which 8 nematode, 3 cestode and one acanthocephalan species were encountered. The metacestode infection, occurring mostly in the liver, showed a high prevalence in *R. norvegicus* (>33%), while the prevalence of *Capillaria hepatica* infection was the highest in *R. nitidus*. All the parasite reported in the present study have been redescribed in brief and their morphometric details, given.

- All the parasites reported in the present studies constitute the first report from Mizoram, Northeast India, as studies on the parasite fauna of rodents have been undertaken for the first time.

- Among the rodent hosts, *Rattus nitidus, Berylmys mackenziei, B. bowersi, Niviventer fulvescens* and *Cannomys badius* constitute a new host record for several parasite species.

- Analysis of the sex-wise prevalence of parasite revealed that prevalence of cestodes infection was higher in female than male hosts excepting *Mus musculus*. for nematodes the prevalence of infection was found to be higher in female hosts excepting *B. bowersi* and *M. musculus.*

- Analysis of locality-wise infection shows that the prevalence of cestode infection was much higher in case of the hosts collected from urban areas excepting *M. musculus,* whereas, the nematode infection was found to be higher in majority of the hosts collected from rural areas except in *B. mackenziei*, *B. bengalensis* and *M. musculus.*

II. Molecular characterization of metacestode of *Taenia* species and *Hymenolepis diminuta*:

During the survey a metacestode of *Taenia* sp. commonly inhabiting the liver lobe and adult tapeworm *Hymenolepis diminuta* occurring in the intestine were frequently encountered. Owing to their frequent occurrence, the present study was extended to provide molecular characterization of these parasites so as to supplement their morphological criteria.

- Genomic DNA of the parasite was isolated using standard ethanol precipitation technique, the rDNA region spanning the ITS1, ITS2 and mitochondrial COI were amplified by PCR following standard procedure. Using the universal primers for trematode species. PCR amplification was done and the PCR product was purified using Genei Quick PCR Purification Kit and sequenced in both directions on an automated sequencer. Sequence analysis was carried out using various bioinformatics tool e.g., BLAST, ClustalW, MEGA etc. ITS2 secondary structures of the cestodes were folded with the help of MFold. The secondary structure in Vienna (dot-bracket-dot) format was used as an input for multiple alignment RNA to calculate sequence structure multiple alignment.

- The sequences obtained were deposited in GenBank with Accession numbers:
  i) **FJ939132-** ITS2 of *Hymenolepis diminuta*
  ii) **FJ939133-** ITS2 of *Taenia taeniaeformis*
  iii) **FJ939134-** ITS1 of *Taenia taeniaeformis*
  iv) **FJ939135-** CO1 of *Taenia taeniaeformis*

- **Metacestode:** PCR amplification of the three regions showed a single band of size 744bp for ITS2, 443bp for ITS1 and 373bp for COI. The sequence analysis results showed that the sequences of the metacestode are closer to those of species of *Taenia* sp., with maximum similarity to *Taenia taeniaeformis.*

- In pairwise alignment, though the ITS sequences and flanking regions of the query sequences with sequences of *Taenia taeniaeformis* (Hyderabad India isolate) showed the presence of 6.4% mismatches for ITS2 and 14.3% mismatches for ITS1, COI sequences showed the presence of only 2.1% mismatches with no gap with those of the three different geographical isolates.

- Phylogenetic trees obtained through NJ and MP methods emerged to be quiet similar placing both the *Taenia taeniaeformis* and the query sequences in the same clade; the boostrap values of (90% and above) of the nodes within the same species were large in all the three regions.

Morphological similarities supplemented by molecular characterization confirmed the metacestode to be *Taenia taeniaeformis.*

- ***Hymenolepis diminuta*:** PCR amplification of ITS2 region showed a single band of size 455bp. The BLAST and multiple alignment analyses showed that the query sequences are closer to those of *H. diminuta.* In phylogenetic analysis, the topology of the trees obtained for ITS2 turned out to be quiet similar, placing the *H. diminuta* isolates from Japan, India, USA and Australia in the same clade, with high bootstsrap value of 99% for both NJ and MP trees.

  Thus, morphological similarities supplemented by close matching of the ITS2 region confirm that **the cestode that commonly inhabits the intestine of the rodent hosts is indeed *H. diminuta*.**

- In RNA Secondary structure analysis, ITS2 of *T. taeniaeformis* and *H. diminuta* showed distinct hallmark of a core secondary structure with four helices, helix III being the longest, an UGGU motif in the 5' end and U-U mismatch in the second helix. The GC content of the metacestode (*Taenia* *taeniaeformis* Accession no. FJ 939133.1) was found to be 61.6%, whereas for *H. diminuta* (FJ939132.1) it was as low as 43.7%.

III. Hepatic histopathological studies in metacestode and *Capillaria*- infected rodents:

Among the helminth parasites recovered during the survey *Capillaria hepatica* and the metacestode of *Taenia* sp. showed a considerably high prevalence, even upto 50% in several of the host species. In view of the frequent occurrence of the two parasites in the rodents collected, a histopathological approach was adopted to find out the effect of these two parasites on the liver of the hosts and to ascertain their potential as a tool for biological control of rodents. The presence of these two parasites altered the normal morphology of the liver. The presence of the metacestode cyst in the liver causes necrosis of the liver tissue and a thin white streak appears on the liver due to the presence of *C. hepatica.*

- The cells appeared spindle shaped with abnormal nuclei around the area where the metacestode occurs. With Masson's trichrome stain, numerous neoplastic cells were observed with abundant collagen sheaths.
- In *C. hepatica* infected liver the lipid content was found to be more than the uninfected liver; partially calcified worm debris were found in the area where the worm disintegrate. Granulomatous lesion surrounded the eggs of *C. hepatica* and septal formations were evident within the infected liver.
- Abundant eosinophilic cytoplasm was observed in the region where both the parasites occurs adjacently.

Considering the damaging effects of the two parasites on the liver tissue, further studies are warranted to ascertain the potential of *Capillaria hepatica* and metacestode of *Taenia* as a tool for biological control for rodent pests.

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BIO-DATA

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Achievements:

- **Received best poster presentation during 19th National Congress of Parasitology and International Symposium on Parasitic Diseases of Animals and Man** held at Department of Zoology, Andhra University, Visakhapatnam, 26-28th October, 2007.
- **Received best poster presentation during 20th National Congress of Parasitology** held at North Eastern Hill University, Shillong, 2008.

- **Received best poster presentation during 21st National Congress of Parasitology: Education and Research in Parasitology in the service of Mankind** held at Panjab University, Chandigarh, 14-16th November, 2009.

Research Publications:

1. **Malsawmtluangi, C.** and Tandon, V. 2009. Occurrence of the metacestode of *Taenia* sp. And the nematode, *Capillaria hepatica* in liver of wild rodents from bamboo growing areas of Mizoram. pp 127-136. In: Tandon, V., Yadav, A.K. and Roy, B. (eds). ***Current Trends in Parasitology*** 20th NCP Proceedings, India; November 3-5, 2008. Panima Publishing, New Delhi.
2. **Malsawmtluangi, C.** and Tandon, V. 2009. Helminth parasite spectrum in rodent hosts from bamboo growing areas of Mizoram, Northeast India. ***Journal of Parasitic Diseases.* 33 (1&2): 28-35.**
3. Tandon, V., Biswal, D. K., Prasad, P. K. and **Malsawmtluangi, C.** 2010. Systematic position and phylogenetic relationships of the cyclophyllidean cestodes: an in-silico study using ITS2 rDNA and sequence-structure alignment. **Paper no. 10. International Conference on Bioinformatics, BIOSTEC, 20-23 Jan, 2010, Valencia. Spain.**
4. **Malsawmtluangi, C.**, Tandon, V and Prasad, P. K. 2010. Morphological and molecular identification of the metacestode parasitizing the liver of rodent hosts in bamboo growing areas of Mizoram, Northeast India. **(Communicated to Helminthologia –** Manuscript submission number HE 0035/2010**)**

Communication in book:

- Biomedical Engineering Systems and Technologies 2010. Tandon, V., Biswal, D. K., Prasad, P. K. and **Malsawmtluangi, C.** Reconstructing the Phylogenetic Relationships of the Cyclophyllidean Cestodes: A case study using ITS2 rDNA and Sequence Structure Alignment. A Fred, J Filipe and H Gamboa (Eds). Communication in Computer and Information Science.

Workshop/Training Course/Seminar/Conference attended:

- **19th National Congress of Parasitology and International Symposium on Parasitic Diseases of Animals and Man** held at Department of Zoology, Andhra University, Visakhapatnam, 26-28th October, 2007.
- **National Symposium on Advances in Zoology: Faunal Diversity and Ecophysiology** held at Department of Zoology, NEHU, Shillong 13-14th March 2008.
- **20th National Congress of Parasitology**, held at North-Eastern Hill University, Shillong, 3rd-5th November, 2008.

- **96th Indian Science Congress,** held at North-Eastern Hill University, Shillong, 3rd - 7th January, 2009.
- "**Insilico Approach to Genome Analysis"** 16th DBT- sponsored training course at Bioinformatics Centre, NEHU, Shillong, 5-11th February, 2009.
- **21st National Congress of Parasitology**, held at Punjab University, Chandigarh. 14 - 16th November 2009.

Conference Presentations:

Oral:

- **Malsawmtluangi C. and Tandon V**. "Spectrum of helminth parasites in rodent hosts from bamboo growing areas of Mizoram" **'National Symposium on Advances in Zoology: Faunal Diversity and Ecophysiology',** NEHU, Shillong, 13-14 March, 2008.

Poster:

- **Malsawmtluangi C. and Tandon V**. "Endoparasitic helminth faunal spectrum in rodent hosts from bamboo growing areas of Mizoram". **19th National Congress of Parasitology**, Visakhapatnam, 26-28 September, 2007.
- **Malsawmtluangi C. and Tandon V.** "Occurrence of the metacestode of *Taenia* sp. And the nematode, *Capillaria hepatica* in liver of wild rodents from bamboo growing areas of Mizoram". **20th National Congress of Parasitology,** North Eastern Hill University, 3rd-5th November, 2007.
- **Malsawmtluangi C and Tandon V.** Morphological and molecular identification of the metacestode parasitizing the liver of rodents hosts in bamboo growing areas of Mizoram, Northeast India. **21st National Congress of Parasitology,** Panjab University, Chandigarh, 14-16th November, 2009.

Sequences submitted to Genbank and accession numbers received:

- **FJ939132-** *Hymenolepis diminuta* adult 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.
- **FJ939133-** *Taenia taeniaeformis* 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.
- **FJ939134-** *Taenia taeniaeformis* adult internal transcribed spacer, partial sequence.
- **FJ939135-** *Taenia taeniaeformis* cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial.