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*. 

119
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*. 

120
- 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 bootstrap 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 bootstrap 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.