STUDIES ON THE DIVERSITY OF HELMINTH PARASITES IN EDIBLE FISHES OF MANIPUR

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2018

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ABSTRACT

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

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ABSTRACT

Fish is an important component of human diet. The people of Manipur extensively consume fish as a stable food. Parasites in fish are a common natural occurrence. Among the parasites, helminths are known to have an adverse effect on the hosts and some of them are also well known for their zoonotic potential. However, reports on the study of helminths in fishes from this region are limited.

The present work was undertaken to study the species composition, identification of the helminths and to evaluate the seasonal variation in prevalence, abundance and mean intensity of helminths occurring in the common edible fishes of Manipur. For identification and characterization, light microscopic and scanning electron microscopic study of helminth parasites, and gene sequencing for three commonly used molecular markers, i.e., nuclear ribosomal internal transcribed spacer 2 (rDNA-ITS2), 18S (or small subunit- SSU) and mitochondrial cytochrome c oxidase subunit 1 (mtCO1) were carried out. The quantitative descriptors like prevalence, abundance and mean intensity were estimated using the methods formulated by American Society of Parasitologists.

A total of 10 species of piscine hosts were collected from different localities of 5 districts of Manipur. The different species of host sampled during the study include, *Channa striata, C. punctata, C. gachua* (Channiformes); *Anabas testiduneus, Trichogaster fasciata* (Perciformes); *Monopterus cuchia* (Synbranchiformes)*; Lepidocephalichthys guntea,* (Cypriniformes); *Clarias*

magur, *Heteropneustes fossilis* (Siluriformes) and *Notopterus notopterus* (Osteoglossiformes). The helminth parasites recovered from these various hosts includes six different species of nematodes (*Camallanus anabantis*, *Neocamallanus singhi*, *Paracamallanus ophiocephali*, *Paraquimperia manipurensis*, *Procamallanus* sp., and *Anisakis* sp.), six species of cestodes (*Lytocestus attenuatus*, *L. indicus*, *L. filiformis*, *L. longicollis*, *Djombangia penetrans* and *Senga lucknowensis*), four species of trematodes (*Astiotrema reniferum*, *Clinostomum philippinense*, *Phyllodistomum sp.* and *Posthodiplostomum* sp.) and four species of acanthocephalan (*Pallisentis ophiocephali*, *P. indicus*, *Pallisentis* sp. and *Echinorhynchus* sp.). This study reports the occurrence of twenty species of helminth parasites, out of which seven species (*Neocamallanus singhi, Paracamallus ophiocephali, Anisakis* sp*., Senga lucknowensis, Clinostomum philippinense, Phyllodistomum* sp. and *Echinorhynchus* sp.) are reported for the first time from Manipur. Moreover, the surface microtopography of *Neocamallanus singhi, Paraquimperia manipurensis, Senga lucknowensis* and *Clinostomum philippinense* are reported for the first time.

Five species of helminths which could not be identified through morphological studies were subjected to molecular methods. The generated sequences were submitted to Genbank and their accession numbers acquired: KX758630, MF947448, MG948466, MG9484667, MF437352, KU761847 and KX758631. The ITS2 sequence of *Clinostomum* species analyses revealed similarity with the African isolate of *Clinostomum tilapiae* and *Clinostomum* sp. of Nigeria and China. Interestingly, the mtCO1 sequence and phylogenetic analyses

portrayed it to be highly identical to *C. philippinense*. The molecular sequence analyses of the digenetic metacercaria bearing the accession number MG948466 and MG9484667 suggested it to be a representative of the genus *Phyllodistomum*. The exact position of the species for this particular metacercaria was not possible since it is not conspecific with any of the species available in the public domain. The 18S sequence of the *Lytocestus* species showed maximum identity with the *Lytocestus indicus* of Indian isolate (99.4%) and similar result is observed in the phylogenetic tree with the nodal support of 100% Bpp values. 18S analyses of the *Senga* species showed maximum homology with *Senga lucknowensis* of Vietnamese isolate with 99.8% similarity and the 18S sequence of the acanthocephala (MF437351) showed that the parasite belong to *Pallisentis* sp. Additionally, a brief description of ITS2 secondary structures of *Clinostomum philippinese* and *Phyllodistomum* sp. have also been discussed.

The host-wise study of prevalence, abundance and mean intensity were observed highest in pre-monsoon in case of *A. testiduneus*, *C. striata* and *C. punctata* whereas, in *C. magur*, prevalence was highest in monsoon and abundance and mean intensity was highest in post-monsoon. The parasite wise study showed the lowest infection level in *L. attenuatus* (9.93%) during pre-monsoon. The highest prevalence of cestodes (*Lytocestus attenuatus, L. indicus, L. filiformes, L. longicollis* and *Djombangia penetrans*) were observed during monsoon. The larval forms of the taxa Lytocestidae were found highest during the end of post-monsoon and onset of pre-monsoon indicating that the peak recruitment occurred during this period, giving rise in the parasite abundance

and mean intensity during monsoon and post-monsoon season. In case of nematodes (*Camallanus anabantis*, *Paraquimperia manipurensis* and *Neocamallanus singhi*) and acanthocephalans (*Pallisentis ophiocephali* and *P. indicus*) the prevalence were observed highest during pre-monsoon.

From the present study, it is revealed that among the fish-borne helminth parasites, *Clinostomum* metacercariae and *Anisakis* sp. emerged as the potential zoonotic trematode prevailing in Manipur, North East India. A temperature range of 24-27˚C revealed to be favorable for infection of helminth parasites. Therefore, we suggest that treatments should be provided during the monsoon and pre-monsoon seasons, which might prove to be effective in controlling the helminth infections.

Key words: Morphological, Light microscopy, Scanning electron microscopy, Molecular, rDNA ITS2, 18S, mtCO1, Secondary structure, Helminths, Nematodes, Cestodes, Trematodes, Acnthocephalan, Seasonal variation, Prevalence, Abundance, Mean intensity.

General Introduction

Fish is indispensable for a large portion of human population in terms of food security and economy. It is a high-protein and low-fat food and is also the most affordable animal protein available to the poor (Belton and Thilsted, 2014). Around 250 million people's livelihood depends directly or indirectly on fisheries and aquaculture (FAO, 2016). It is also an object of sport and pets in the case of ornamental fish. Expansion and growth in fishery and aquaculture during the last decades has drawn the attention to the problems posed by parasites and their importance in fish health, behavioural change, productivity, and hygiene. Parasitic infection in fishes is very common throughout the globe, which deteriorates mechanical, physical and reproductive system of the hosts (Rogers, 1978).

Majority of fishes carry heavy infection of parasites and that depreciates the food value and may even cause mortality. Moreover, there are some zoonotic helminth parasites that could be transmitted to human beings only through fish (Khanum *et al*., 2008). Helminths damage the host's tissues lining causing microscopic lesions in the tissue and its countermeasure against the host immunity have potential to limit immune function. This enhances the chances for secondary infection by different microbes (Horsnell, 2014). Thus, parasitic infestation of fish in tropical and subtropical countries represents a serious problem for aquaculture due to severe economic losses either directly or indirectly. Apart from the loss incurred in the economy, it is also a concern of human health. Many important helminths cause human zoonotic diseases, which are transmissible to human beings from fishes through eating raw or partially cooked fish.

One the fundamental aspects of understanding the biology, diversity and epidemiology of a parasite lie in proper identification. The need of accurate identification and proper naming of the organism is increasing with the growing understanding of science

(Monis, 1999). It is believed that lack of comprehensive taxonomic accounts of parasites is one of the causes for the crisis of emerging infectious diseases (Brooks and Hoberg, 2006). The phylogenetic studies accompanied by biosystematics contribute better understanding regarding life cycle, transmission dynamics and prognostic framework. Taxonomic studies, for past 250 years were done relying on morphological features of an organism. Morphological identification is no doubt the most simple and straightforward way to identify an organism but many individuals that look physically similar may be totally different in their genetic makeup (Friedheim, 2016). This is where the molecular approach in identification becomes important.

With the progress in genetic and evolutionary studies, the molecular approach in systematics has been widely used. Molecular approaches such as DNA based PCR methods have proven to be useful in identification of parasites up to species level as well as differentiation of closely related helminth parasites (Caira *et al*., 2013; Chaudhary *et al*., 2016). In this context, the nuclear ribosomal internal transcribed spacer 2 (rDNA-ITS2), 18S and mitochondrial gene cytochrome c oxidase subunit 1 (mtCO1) have been extensively used to resolve taxonomic issues and to differentiate closely related parasitic species (Locke *et al*., 2015). Because these genes display rapid rate of evolution, they have emerged as the locus of choice in answering questions related to taxonomy, population genetics, species identification and phylogenetic relationships of various helminth parasite species like trematode, nematode, and cestode. An additional advantage of using ITS2 is the possibility of predicting its secondary structure from the primary sequence data and is known to provide further information that can be useful in delineating closely related species. This approach has been successfully used in discriminating closely related species among plants, fungi and parasitic groups, including cestodes and trematodes (Rampersad,

2014; Ghatani *et al*., 2014; Zhang *et al*., 2015; Sharma *et al*., 2016a, b). The integrated approach of both morphological and molecular studies, therefore, is the universally accepted method for parasite identification (Athokpam and Tandon 2014).

Knowledge of infection of freshwater fishes harbouring different helminth parasites will provide important information regarding epidemiological index giving the status of human infection sources, marketability of the fishes and prevalence of the parasites. It is important to have a complete list of habitat-specific parasites for advance disease management. Therefore a detailed survey needs to be carried out to determine the prevalence, intensity and seasonal variation in the occurrence of different helminth parasites, and also the species composition of helminth parasite spectrum in fishes.

The state of Manipur which forms part of global diversity hotspots belongs to Indo-Burma Biodiversity Hotspot. In this comparatively small geographical area, there is a wide range of climate from temperate to tropical and has a wide range of forests and biodiversity (Singh, 2006). It has four major river basins with rich pisces diversity sheltering more than 200 local species belonging to 84 genera, 31 families and 11 orders out of which 15 are in the endangered list (Vishwanath, 2000; Vishwanath *et al.*, 2007). The four river basins include Barak river basin in the west, Manipur river basin in the central, Yu river basin in the east and Lanye river basin in the west (Haokip, 2007). It also has a total fish production of 19,200 tonnes/yr (Devi *et al*., 2012). Although, there are rich varieties of fishes available in the state, the spectrum of helminth parasites studied in these fishes is scanty. Very limited information is available on helminth parasites of fishes in this region (Shomorendra and Jha, 2003; Gambhir *et al*., 2006; Puinyabati *et al*., 2010).

Therefore, the present study aims to incorporate the following objectives:

1). To examine helminth parasites in some edible fishes of Manipur and to identify the fish-borne helminth parasites of zoonotic potential, if present.

2). To analyze seasonal variation in prevalence, abundance and mean intensity of the most frequently occurring helminth parasites.

REVIEW OF LITERATURE

Fish and fishery product is the most rewarding commodity that has grown significantly and traded internationally with annual sales of about US $80 billion (FAO, 2016). According to reports from the Handbook on Fisheries Statistics (2014), India ranks second in overall fish production, contributing 5.68% to global fish production, where the total fish production of India is 9.58 million metric tonnes (accounting 6.14 million metric tonnes to the inland sector and 3.44 million metric tonnes to marine sector). Over 14.49 million people draw their source of income directly or indirectly from fisheries sector. Therefore, fisheries sector has a stupendous contribution to the socio-economic status of the country. A large number of people from rural community rely on fish for their livelihood as well as food security (Paul and Chakraborty, 2016). The number of people absorbed in fisheries and aquaculture during the past three decades has grown very fast and has become an important source of employment and income in the developing countries (Bennell, 2010).

Fish is considered as the most diverse group among vertebrates totaling up to 33,000 described species (Froese and Pauly, 2012). However, the number of fishes consumed by humans is relatively small. Several research work have been done on fish and showed that the minerals and nutrients present in fish are beneficial and required for functionality of human health (Thilsted *et al*., 2014). The FAO (2016) estimates that about one billion people worldwide rely on fish as their primary source of animal protein. A consistent source of fish is essential for the nutritional and financial health of a large segment of the world's population. Fish consumption reduces the risk of death from coronary heart disease, moreover consumption by women reduces the risk of sub-optimal neuro-development in their offspring and also it may reduce the risk of multiple other

adverse health outcomes, including ischemic stroke, non-fatal coronary heart disease events, congestive heart failure, atrial fibrillation, cognitive decline, depression, anxiety and inflammatory diseases (Tacon and Metian, 2013). They are also often the cheapest and most frequently consumed animal-source food in low-income food deficit countries as a source of protein, fatty acids, essential vitamins and minerals such as vitamin A, calcium, iron, zinc and iodine (World Bank, 2006).

Helminth parasite infection in fishes

Fish, like any other animals and human beings suffer from parasites and diseases. Approximately, 50% of fish diseases particularly of freshwater fishes are caused by different parasites (Rogers, 1978). Helminth parasites comprising of trematodes, cestodes, nematodes, and acanthocephalans are very common among freshwater fishes (Hoffman, 1999). The infection of fish by these helminths and its effect on them has caused a concern in the field of fisheries and aquaculture. Moreover, it raised a problem related to human health, particularly in Asian countries where eating partially cooked fish is a part of food habit. Extensive work on these fish parasites which have been carried out by many authors are as follows:

Cestodes: Cestodes are the dominant endohelminths in elasmobranchs and have only few significant radiations in bony fishes (Caryophyllidea, Proteocephalidea, and Pseudophyllidea) (Cribb *et al*., 2002). Freshwater fishes, particularly teleost in South America are found to be infected by 89 taxa of cestodes from 6 orders, belonging mostly to the order Proteocephalidea (Rego *et al*., 1999). The ecology and pathology of the genus *Proteocephalus* were studied by Amin (1990) from lake fishes in Wisconsin and its life cycle is described by Scholz (1999). Exhaustive work on diversity and distribution of fish tapeworms of the Bothriocephalidea has been carried out by Kuchta *et al*. (2008).

Cestodiasis among freshwater fishes have been studied by different workers in different part of the globe (Oros *et al*., 2010; Bhure and Nanware, 2011; Choudhury *et al*., 2013; Ash *et al*., 2015; Brabec *et al*., 2015; Koiri and Roy, 2017a).

In recent decades, many new taxa of cestodes have been described, especially from India, but very few of the recent studies actually provided reliable data on the morphology and taxonomic status of fish tapeworms (Ash, 2012). Gupta and Sinha, (1984) described *Pliovitellaria osteobramensis* from the intestine of the cyprinid fish *Osteobrama cotio* from Lucknow, India. The following caryophyllidean taxa described from Indian cyprinid fishes by different workers were considered to be valid by Oros *et al* (2012): *Adenoscolex oreini*, *Breviscolex aurangabadensis*, *B. Naldurgensis Paracaryophyllaeus osteobramensis* and *Paracaryophyllaeus lepidocephali*. But in Northeast India, the most common cestodes found parasitizing freshwater fishes are *Lytocestus* of the order Caryophyllidea and *Senga* of the order Bothriocephalidea. Four new species of the genus Lytocestus (*L. attenuatus, L. clariae, L. heteropneustii and L. assamensis*) were described from this region by Tandon *et al*. (2005) from Catfishes of Guwahati, Assam and Shella, Meghalaya. Occurrence of other species include (Lytocestidae): *L. indicus, L. filiformes, L. longicollis, L. fossilis, L. birmanicus* and *Djombangia penetrans*; (Bothriocephalidae): *Senga sp*. (Chakravarty and Tandon, 1988; Jyrwa *et al*., 2016; Malsawmtluangi and Lalramliana, 2016; Koiri and Roy, 2017b; Devi *et al*., 2017).

Trematodes: The Monogenea are external parasites of all groups of fishes, but have greatest diversity in bony fishes. Whittington (1998) estimated that the world fauna of fishes might ultimately prove to harbour 25000 species of monogeneans of which just 3000–4000 are described so far. The class Monogenea is the most diversified group and contains the largest number of species parasitizing Neotropical fish (Boeger *et al*., 2006).

In most of the cases, monogenean parasites are host-specific, although some may infect several hosts from different families. Monogeneans included in Dactylogyridae are primarily parasites of the gills and so are found on gill filaments, gill rakes or the lateral surfaces of gill arches. *Gyrodactylus* and *Dactylogyrus* are the two most common genera of monogeneans that infect freshwater fish (Klinger and Floyd, 2010).

Digenea, which are essentially internal parasites have their greatest diversity in the teleosts. Most commonly occuring parasites of chondrichthyans are the five families of digeneans (Azygiidae, Gorgoderidae, Ptychogonimidae, Sanguinicolidae and Syncoeliidae) which are in noticeable similarity with the monogeneans. The digenetic trematode metacercarial infection in fishes incurs deleterious effect to fishes and are extensively studied by different authors throughout the world (Lane and Morris, 2000; Nithiuthai *et al*., 2002; Hicks and Steele, 2003; Bullard and Overstreet, 2008; Kelly *et al*., 2010; Shareef and Abidi, 2012; Sohn *et al*., 2015).

Nematodes: Among helminths, nematodes are considered as the most deadly parasites for the fishes (Yasmin and Bilqees, 2007). They are considered to be one of the most diversed in the animal kingdom with as many as 256 families and more than 40,000 species occur in the animal kingdom (Gebremedhn and Tsegay, 2017). Moravec (1995) studied the parasitic nematodes of freshwater fishes of Europe and provided contemporary knowledge of the taxonomy, biology and ecology of the parasites. Nematodes of freshwater fish from the Sudan and Ethiopia was also reported by Moravec and Scholz (2017) where 18 species belonging to the order Ascaridoidea, Camallanidea, Cosmocercoidea, Habronematoidea, Oxyuroidea, Seuratoidea, Trichilloidea and Dioctophymatoidea were recovered. Moravec and his co-workers have extensively contributed in the field of taxonomy and biosystematics of fish nematodes (Moravec and

Justine 2006; Moravec, 2007a,b; Santos and Moravec, 2009; Moravec *et al*., 2012; Moravec and Buron, 2013; Moravec *et al*., 2016; Moravec and Scholz, 2017). Most commonly occurring nematode genus in North American freshwater fishes are recorded to be *Philometra, Philonema, Pseudocapillaria, Cystidicola, Huffmenella,* and *Haplonema* (Hoffman, 1999). Likewise, most commonly occurring nematodes of fishes in India include different species of *Camallanus, Procamallanus, Paracamallanus, Neocamallanus, Paraquimperia, Contracaecum, Spinitectus, Anisakis, Rhabdochona, Philometra, Eustrongylides, Philometroides, Cosmoxynematoides* and *Hysterothylacium* (Lakshmi, 2010; Moravec *et al*., 2012; Das and Goswami, 2014; Jyrwa *et al*., 2016; Fartade *et al*., 2017; Zimik and Roy, 2017).

Acanthocephalans: Acanthocephalans are known to have complex life cycles and complete life cycles have been worked out for only 25 species (Nabi *et al*., 2015), where the number of described species reached up to 1,150 belonging to125 genera and 19 families (Verweyen *et al*., 2011). Plenty of literatures are available on prevalence, seasonal variation in occurrence and population dynamics of different acanthocephalans parasitizing fishes in different parts of the globe (Rauque *et al*., 2003; Paterson *et al*., 2011; Tepe and Oguz 2013; Briones *et al*., 2015; Sheema *et al*., 2015; McAllister *et al*., 2016; Koiri and Roy, 2017c).

Most commonly occurring acanthocephalans among the freshwater fishes of India are *Pallisentis spp.* and *Neoechinorhynchus* spp, of which several species of *Pallisentis* are described as new, particularly from *Channa punctata* from different parts of Uttar Pradesh (Gupta *et al*., 2015a, b; Gautam *et al*., 2017).

Prevalence and seasonal studies

Review of the literature revealed the presence of several reports on seasonal variations and population dynamics of acanthocephalans infecting various fishes from different parts of India. Gupta *et al*. (2012b) worked on on seasonal variation of *Pallisentis* on Chanid fish of Uttar Pradesh and showed that the prevalence rate of the parasite was as high as 100% during February to March. Sheema *et al*. (2015) observed very high rate of prevalence of *Echinorhynchus velli* among fishes during summer. The infection rate of acanthocephala in economically important food fishes of Kashmir was studied by Farooq *et al*. (2016). Sakthivel *et al*. (2014) showed how ecological factor of *Echinorhynchus* influence the population dynamics of the parasites.

Several reports are available about the occurrence of helminth parasites of freshwater fishes in different parts of the globe (Cribb *et al*., 2002; Aguilar *et al*., 2005; Richard *et al*., 2012; Crafford *et al*., 2014). Awharitoma and Okaka (1999) reported that the rate of helminth infection is as high as 60.8% among cichlid fishes of Okhuaihe river of Nigeria. However, Yakabu *et al*. (2002) showed that *Tilapia zilli* and *Clarias gariepinus* inhabiting the river of Uke of Nigeria have 61% of and 55% helminth infection, repectively. Extensive literature is also available on the fish parasites in different parts of India (Pokharel, 1999; Pandey *et al*., 2002; Yousuf *et al*., 2011). According to Vankara *et al.* (2011), 78% of freshwater eel *Mastacembelus armatus* collected from the river Godavari were found to be infected with different helminth parasites. Dhole *et al*. (2010) also reported a high rate of infection in *M. armatus* compared to channid and silurid fishes in Maharashtra. However, Nimbalkar *et al*. (2010) observed that the rate of infection was as high as 100% among the fishes of Jaikwadi Dam, Maharashtra. A high rate of helminthiasis has also been recorded among different fishes in West Bengal and Uttar Pradesh (Kakaji, 1969; Kumar and Kumar, 2013; Ramuda and Dash, 2013; Chakrabarti *et*

al., 2012; Balaji *et al*., 2013). However, only limited information is available about the fish parasites of northeast India, and is mostly restricted to Meghalaya, Arunachal Pradesh, Assam and Tripura (Thapa *et al*., 2009; Tripathi, 2011; Saha *et al*., 2011; Singha *et al*., 2010; Das and Goswami, 2014; Jyrwa *et al*., 2016; Koiri *et al*., 2017a-c).

Manipur shares similar climatic conditions with its neighbouring states, however, having few sporadic report, no systematic account of occurrence of different types of helminths in the state are available in literature (Shomorendra and Jha, 2003; Gambhir *et al*., 2006; Puinyabati *et al*., 2010; Sangeeta *et al*., 2011; Devi *et al*., 2017). It is important to have a complete list of habitat-specific parasites for management of parasitic diseases. Therefore a detailed survey needs to be carried out to determine the prevalence, intensity and seasonal variation in the occurrence of different helminth parasites, and also the species composition of helminth parasite spectrum in fishes.

CHAPTER 1
MORPHOLOGICAL OBSERVATIONS ON HELMINTH PARASITES

1.1 Introduction

Fish are diverse group of vertebrates constituting almost half of the total number of vertebrates in the world and freshwater fishes contribute 25% of the vertebrate species (Winemiller *et al*., 2008). The occurrence of both parasites and microbial infections in fishes are common and the parasitic infections occur at a higher percentage as compared to microbial infections (Rogers, 1978). Among the parasite, the helminths are known to have an adverse effect on the hosts and some of them are also well known for their zoonotic potential. Helminth parasites consist of four major classes, viz., Trematoda, Cestoda, Nematoda and Acanthocephala and the highest number of species with zoonotic potential is believed to be the class Trematoda (Robinson and Dalton, 2009). In food-borne trematodiases, transmissions to human beings occur through aquatic products and they are common in Asian countries. Despite the increasing occurrence and various public health impacts, these diseases are considered as neglected tropical diseases (Chai *et al*., 2009; Keiser and Utzinger, 2009).

In case of fish as a host, evidence indicates that the parasitic infection in nutritionally demanding ones can be associated with host foraging strategies, altering prey preferences and food intake rates (Barber, 2005). The occurrence of helminth parasites in freshwater fishes and its effects (behavioral changes, pathogenicity, immune response, etc.) have been studied by many authors, but there is no real consensus on the taxonomy of many helminths (Simner, 2016). With the advancement in technology, the importance of systematics and taxonomy has been realized by many biologists, the basic principles of

which are to combine various types of data to produce classifications reflecting the natural history of living organisms. It is pivotal to accurately classify the parasites, not only from a taxonomic point of view but also because they provide a framework around which all aspects of parasite's biology can be studied (Monis, 1999).

The study of helminth parasite fauna in respect to its taxonomy have been reported from different parts of the world (Khalil *et al*., 1996; Moravec, 2007a; Oros *et al*., 2010; Verweyen *et al*., 2011; Caffara *et al*., 2017). Many new parasite species of fishes have also been described from different parts of India contributing to the field of parasitology (Lakshmi, 2010; Bhure and Nanware, 2011; Nimbalkar *et al*., 2013; Vankara *et al*., 2014; Gupta *et al*., 2015).Various authors have also studied the occurrence and spectrum of helminths of fishes in Northeast India (Tandon *et al*., 2005; Jyrwa *et al*., 2016; Devi *et al*., 2017; Koiri and Roy, 2017a-c).

Manipur is believed to be a home for more than 200 species of fishes. However, very limited and sporadic literature is available on taxonomy and systematics of ichthyoparasites (Sangeeta *et al*., 2011; Puinyabati *et al*., 2015; Devi *et al*., 2017). There is a possibility that thorough investigation with the help of various methods (including molecular tools), may lead to description of some unknown parasites/new locality record in this state of northeast India. Therefore, the present work was undertaken to examine the species composition and identify the helminth parasites in some edible fish and to delineate parasites of zoonotic potential, if present.

1.2 Materials and method

Study area and collection of host

Manipur is situated in the north-eastern region of India, covering 22,327 square kilometers (24° 44' N and 93° 58' E). It has four major river basins with a rich pisces diversity sheltering more than 200 local species (Vishwanath *et al*., 2007). The four river basins include Barak river basin in the west, Manipur river basin in the central, Yu river basin in the east and Lanye river basin in the west. The host collections were done every alternate month for three years (Number of sampling = 18 i.e., from August 2014 - July 2017) from various water bodies (rivers, lakes, ponds and streams). The fishes were procured from fishermen (where they caught it from water body using various fishing gears, mostly gill nets, lift nets and cast nets) and from the fishermen selling fish in the local fish markets under five districts: Ukhrul (Kachai and Hungpung), Imphal East (Lamlong), Imphal West (Lamphel), Thoubal (Kakching, Tengtha) and Bishnupur (Loktak, Moirang and Ningthoukong) (Fig. 1.1).

The piscine host sampled during the study includes *Channa striata*, *C. punctata*, *C. gachua* (Channiformes); *Anabas testudineus, Trichogaster fasciata* (Perciformes); *Monopterus cuchia* (Synbranchiformes); *Lepidocephalichthys guntea,* (Cypriniformes); *Clarias magur, Heteropneustes fossilis* (Siluriformes) and *Notopterus notopterus* (Osteoglossiformes) (Fig.1.2). The fishes were identified following Vishwanath *et al*. (2007).

Examination of host for recovery of helminth parasites

Fishes collected from different sites were bought alive and examined thoroughly for recovery of helminth parasites. To recover ectoparasites, the external body and organs such as eyes, gills, scales, fins and mouth were thoroughly examined with the help of a magnifying glass. The host fishes were then dissected and peritoneal lining of the body cavity, the internal organs such as heart, lungs, livers, stomach, intestine, swim bladder, gall bladder, spleen, kidney, gonads, viscera, mesenteries and muscles were observed to recover endoparasites. The collected parasites were counted, recorded and processed for identification.

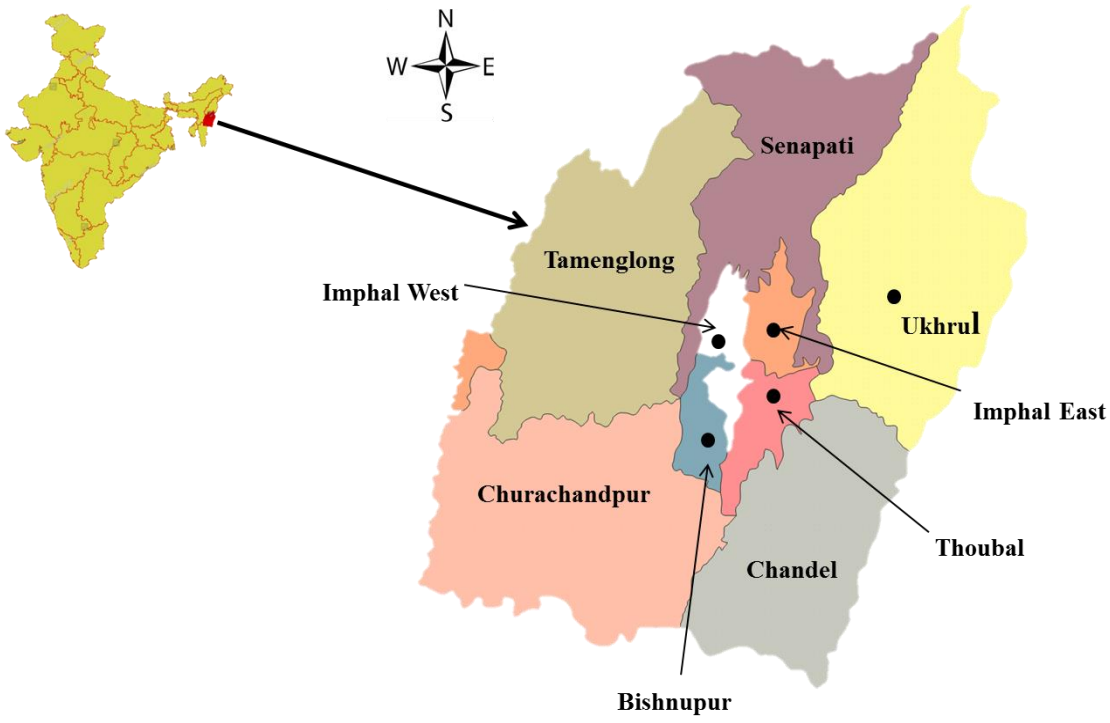

Fig. 1.1 Map of Manipur showing the different sampling sites surveyed for the collection of fish hosts (●)

[Map source: https://d-maps.com/carte.php?num_car=31958&lang=en]

Channa striata

Channa punctata

Channa gachua

Trichogaster fasciata

Clarias magur

Anabas testudineus

Heteropneustes fossilis

Monopterus cuchia

Notopterus notopterus

Lepidocephalichthys guntea

Fig. 1.2 Fish hosts collected from different localities during the study period

Light microscopy (LM)

Nematodes and Acanthocephalans: The freshly sampled worms were washed in PBS (Phosphate buffered saline) and fixed in 70% ethanol. For permanent slide preparation, the nematodes were cleared in ascending grades of glycerine (30%, 50%, 70%, 80%, 90% and 100%) and finally mounted using Kaiser's glycerine jelly (50 ml water + 80 g gelatin + 50 ml glycerol + 0.1 g phenol). Temporary slides were also prepared by immersing the parasites in lactophenol (20 ml lactic acid + 20 ml phenol + 20 ml distilled water + 40 ml glycerine) overnight and temporarily mounted in lactophenol (Soulsby, 1982). The prepared slides were viewed under Leica Microscope (DM1000) and images were captured.

Cestodes and Trematodes: The platyhelminthes collected were washed in PBS. The washed worms were flattened by placing them between the slides and fixed overnight/few days (depending on the size and thickness of the parasites) in 10% formalin. After fixation, whole mount preparation was done by staining in Borax carmine (destained in acid water for the parasites that were overstained), dehydrated in graded series of alcohol (30%, 50%, 70%, 80%, 90% and 100%), cleared in methyl benzoate and mounted in Canada balsam (Soulsby, 1982). The prepared slides were viewed under Leica Microscope (DM1000) and images were captured.

Identification

Identification of parasites through morphological characters involving light microscope was carried out following standard literature (Yamaguti 1959, 1961, 1963, 1971); Keys to the Trematoda Vols. 1-3 (Gibson *et al*., 2002; Jones *et al*., 2005 and Bray

et al., 2008); CIH Keys to the Cestode Parasites of Vertebrates (Khalil *et al*., 1994) and CIH Keys to nematodes nos. I-X (Anderson *et al*., 1974-1983).

1.3 Observation and Results

In the present study, six different species of nematodes, six species of cestodes, four species of trematodes and four species of acanthocephala infecting various edible fishes of Manipur were studied and discussed. All the parasites are illustrated with a brief description, morphometric measurements (in tabular form) and remarks for each of them as follows:

Class: Nematoda

Subclass: Secernentea

Order: Spiruridea

Family: Camallanidae Railliet and Henry, 1915

Genus: *Camallanus* Railliet and Henry, 1915

Species: *C. anabantis* Pearse 1933

(Fig. 1.3) (Table 1.1)

Total number of specimens collected: 660

Description: mouth slit-like, with 9 longitudinal ridges embellished with beaded structure; buccal capsule consisting of two lateral chitinous valves, with longitudinal rib like thickenings internally. From the point of junction of the valves, dorsally and ventrally, a trident-shaped chitinous process is directed backwards; a pair of sclerotized plate present in the anterior portion of sub-lateral region. Esophagus consisting of a short anterior muscular portion and a long posterior glandular portion enlarged posteriorly. Tail ends in bifurcated or trifurcated mucrons. Male: Caudal alae absent, spicules unequal and feebly chitinized; gubernaculum absent. Female: vulva present at about middle of the body, posterior ovary lacking, viviparous.

Host: *Anabas testudineus*

Habitat of the parasite: Intestine

Habitat of the host: Ponds, canals and lakes.

Locality: Imphal East (24.7807° N and 93.9674° E), Imphal West (24.7828° N and 93.8859° E), Thoubal (24.7828° N and 93.8859° E) and Bishnupur (23.0679° N and 87.3165° E)

Sampling Date: 1-15 of every alternate month from August 2014 - July 2017

Remarks: Railliet and Henry, (1915) first created the genus *Camallanus*. The genus *Camallanus* consists of 19 species inhabiting different hosts (Yamaguti, 1961). Numerous works on the genus *Camallanus* in fish hosts have been carried out by various authors like Moravec and Scholz (1991), De and Maity (1995), Levsen and Berland (2002), Kuzmin *et al*. (2011), Deepananda (2013), Mascarenhas and Müller (2017). *Camallanus anabantis* was first described from *Anabas testudineus* from Siam and *Clarias magur* from India by Pearse but it was later redescribed by Chakravorty (1939). This nematode is also found to be harbored by *Trichogaster microlepis* (Yooyen *et.al*.,2006). This nematode has also been previously reported from Assam and Manipur by Puinyabati *et al*. (2015).

Table 1.1 Morphometric measurements of *Camallanus anabantis* collected from Manipur, India (N= 10)

Characters	Range (in mm)	Mean ± Standard Deviation
Body		
Length	6.00-13.00	9.50±4.949
Breadth	0.45-0.52	0.485±0.049
Buccal capsule		
Length	0.08-0.10	0.090±0.01
Breadth	0.12-0.15	0.135±0.021
Muscular oesophagus	1.10-1.20	1.150±0.070
Glandular oesophagus	1.80-2.12	1.960±0.226
Anterior nerve ring	0.18-0.20	0.190±0.014
Vulva length	0.04-0.05	0.045±0.007
Vulvar breadth	0.05-0.06	0.055±0.007
Tridents		
Length	0.02-0.03	0.025±0.007
Breadth	0.01.0.02	0.015±0.007
Sclerotised plate		
Length	0.04-0.05	0.045±0.007

Breadth	0.09-0.11	0.100±0.014
Mucron	0.04-0.06	0.050±0.014

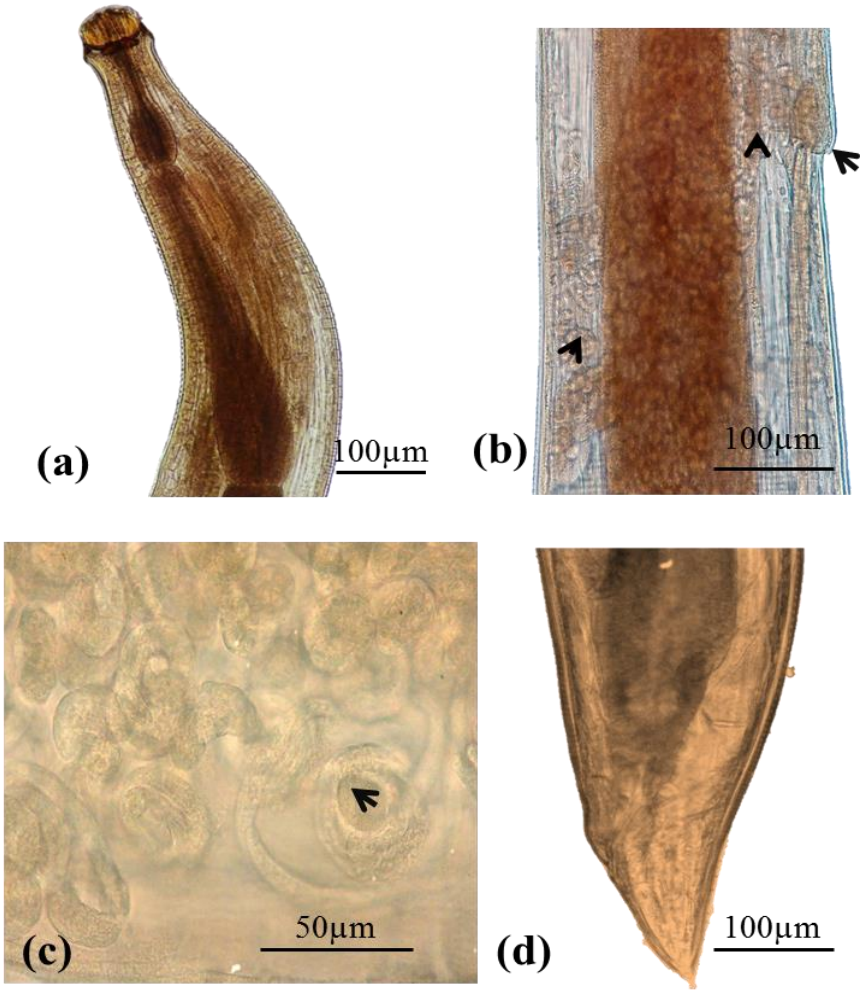

Fig. 1.3 Light microscopic (Leica DM1000) images of *Camallanus anabantis*

a) Anterior end

b) Gravid female showing the presence vulva (arrow) and larvae inside uterus (arrowheads)

c) Magnified view of larvae inside uterus (arrow)

d) Tail

Genus: ***Neocamallanus***

Species: ***N. singhi*** **Ali, 1957**

(Fig. 1.4) (Table 1.2)

Total number of specimens collected: 367

Description: Mouth elongated dorso-ventally; lips absent; buccal capsule consisting of two lateral valves; trident absent. Well-developed sclerotized plate present behind the valves, sub-laterally attached without a thread or rod like structure. Esophagus consisting of a short anterior muscular portion and a long posterior glandular portion enlarged posteriorly. Cuticle striated transversely. Male: Caudal alae well developed with several pairs of pedunculatated and sessile papillae. Female: Vulva equitorial, eggs embyonated.

Host: *Anabas testudineus*

Habitat of the parasite: Intestine

Habitat of the host: Ponds, canals and lakes

Locality: Imphal East (24.7807° N and 93.9674° E), Imphal West (24.7828° N and 93.8859° E), Thoubal (24.7828° N and 93.8859° E) and Bishnupur (23.0679° N and 87.3165° E).

Sampling Date: 1-15 of every alternate month from August 2014 - July 2017

Remarks: The genus *Neocamallanus* was erected by Ali (1957) with type species *N. singhi* recovered from *Channa punctata* from Hyderabad, India. Though many authors

synonymized *Neocamallanus* with *Camallanus* (Yeh, 1960), Moravec and Sey (1988), and Yamaguti (1961) accepted this genus as valid. The life cycle of this genus was studied by De *et al*. (1984) and *N. singhi* was also redescribed by De and Majumdar (1984). This is the first record of the species from Manipur, India.

Table 1.2 Morphometric measurements of *Neocamallanus singhi* collected from Manipur, India (N= 10)

Chararacters	Range (in mm)	Mean± Standard deviation
Body		
Length	3.34-4.11	3.725±0.544
Breadth	0.17-0.18	0.175±0.007
Buccal capsule		
Length	0.07-0.08	0.075±0.007
Breadth	0.08-0.09	0.085±0.007
Muscular oesophagus	0.34-0.36	0.350±0.014
Glandular oesophagus	0.50-0.60	0.550±0.070
Sclerotised plate		
Length	0.02-0.03	0.025±0.007
Breadth	0.05-0.06	0.055±0.007
Spicules	0.037-0.40	0.218±0.256

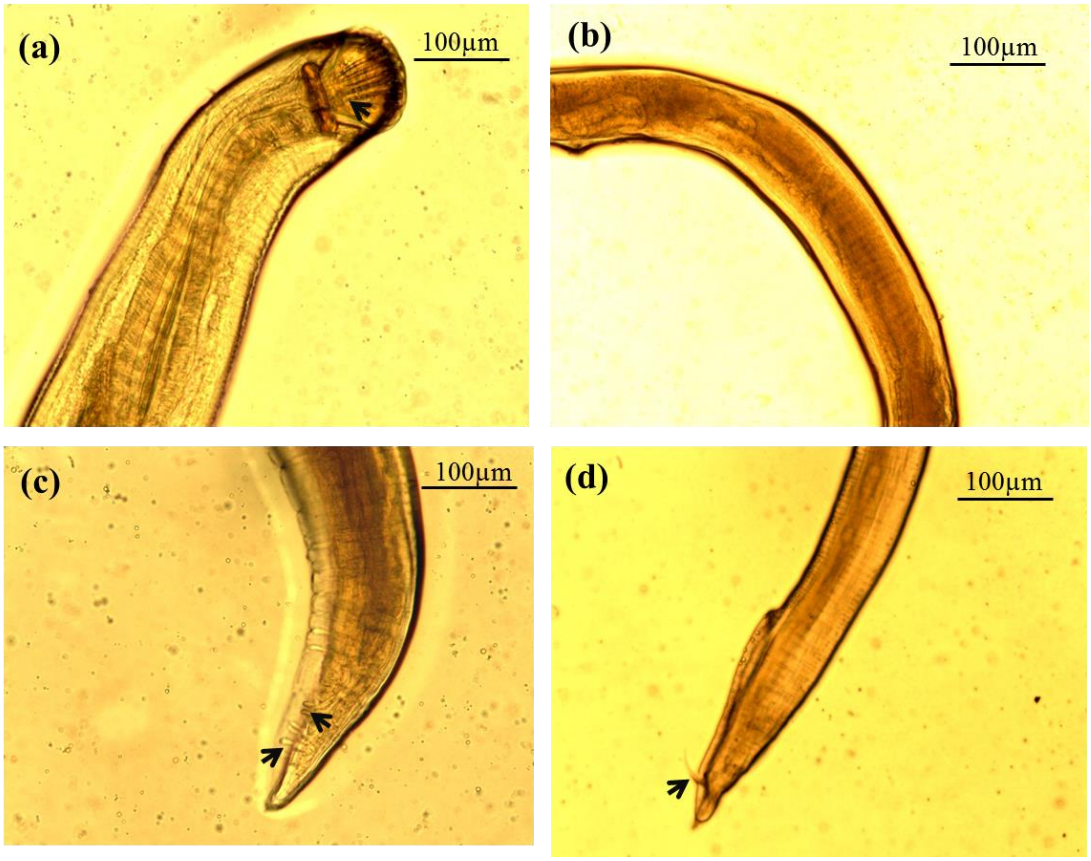

Fig. 1.4 Light microscopic (Leica DM1000) images of *Neocamallanus singhi*

a) Anterior end showing buccal cavity (arrow)

b) Lateral view of the body

c) Tail end of male showing caudal pedunculated papilla (arrows)

d) Tail end of male showing spicule (arrow)

Genus: *Paracamallanus*

Species: *P. ophiocephali* Yorke and Maplestone, 1926

(Fig. 1.5) (Table 1.3)

Total number of specimens collected: 26

Description: Mouth elongated dorso-ventrally; large chitinous teeth present in the buccal cavity. From the point of junction of the valves, dorsally and ventrally, a trident-shaped chitinous process is directed backwards; sclerotized plate absent. Cephalic papillae not visible; deirids present. Esophagus consisting of a short anterior muscular portion and a long posterior glandular portion enlarged posteriorly. Tail conical, mucron not divided. Male: Caudal alae absent, spicules unequal and feebly chitinized; gubernaculum absent. Female: Vulva about middle of the body, posterior ovary lacking, viviparous.

Host: *Anabas testudineus*

Habitat of the parasite: Intestine

Habitat of the host: Ponds, canals and lakes

Locality: Imphal East (24.7807° N and 93.9674° E), Imphal West (24.7828° N and 93.8859° E), Thoubal (24.7828° N and 93.8859° E) and Bishnupur (23.0679° N and 87.3165° E).

Sampling date: 1-15 of every alternate month from August 2014 - July 2017

Remarks: Yorke and Maplestone (1926) separated *Paracamallanus* from *Camallanus* because of having a large chitinous buccal cavity or pharynx behind buccal valves in *Paracamallanus*. Yamaguti (1961) also considered *Paracamallanus* as a separate genus. *Paracamallanus* and *Camallanus* share some similar morphological features but the differences among them are huge enough to place them in separate genus (Zimik and Roy,

2017). This parasite has also been reported from Meghalaya by Jyrwa *et al*. (2016). The present study is the first report of the occurrence of *P. ophiocephali* from Manipur, India.

Table 1.3 Morphometric measurements of *Paracamallanus ophiocephali* collected from Manipur, India (N= 10)

Characters	Range (in mm)	Mean± Standard deviation
Body		
Length	5.00-11.00	8.000±4.242
Breadth	0.43-0.50	0.465±0.049
Buccal cavity		
Length	0.12-0.13	0.125±0.007
Breadth	0.14-0.16	0.150±0.014
Anterior oesophagus	0.40-0.45	0.425±0.035
Posterior oesophagus	1.10-1.13	1.115±0.021
Sclerotized plate		
Length	0.03-0.04	0.035±0.007
Breadth	0.10-0.11	0.105±0.007
Mucron	0.05-0.06	0.055±0.007

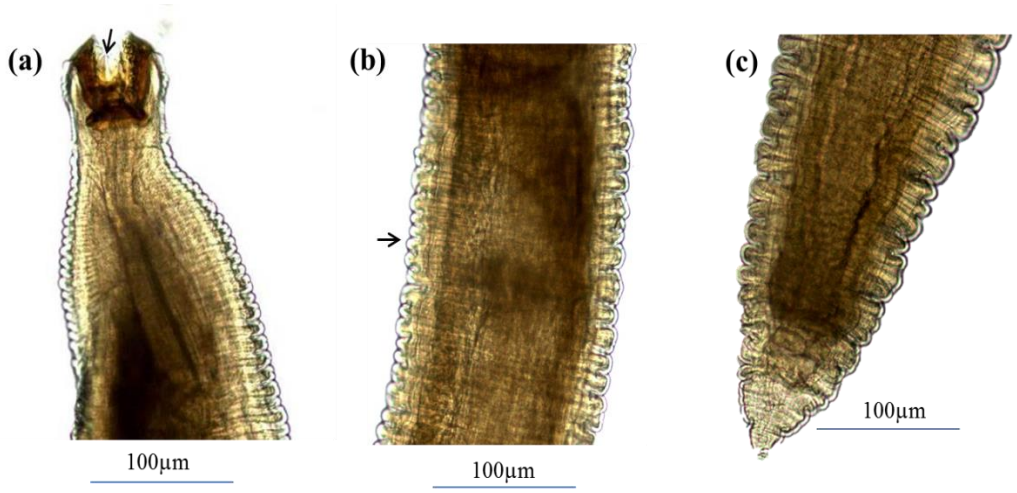

Fig. 1.5 Light microscopic (Leica DM1000) images of *Paracamallanus ophiocephali*

a) Anterior end showing chitinous teeth (arrow)

b) Lateral view of the body showing annulation (arrow)

c) Tail end of female

Order: Ascaridida

Family: Quimperiidae Baylis, 1930

Genus: *Paraquimperia* Baylis, 1934

Species*: P. manipurensis* Shomorendra and Jha, 2003

(Fig. 1.6) (Table 1.4)

Total number of specimens collected: 477

Description: Body small, slender, anterior end curved dorsally; apical region ends in mouth bounded by three lips; buccal cavity absent. Cervical alae present and extend up to the level of anterior esophageal corpus; oesophagus cylindrical with longer and smaller anterior portion, and shorter and wider posterior region; caudal alae absent. Male: Tail tapered to a fine point; several numbers of papillae present. Spicules equal, and crescent-shaped. Female: vulva with two prominent lips present at posterior third of the body; eggs spherical.

Host: *Anabas testudineus*

Habitat of the parasite: Intestine

Habitat of the host: Ponds, canals and lakes

Locality: Imphal East (24.7807° N and 93.9674° E), Imphal West (24.7828° N and 93.8859° E), Thoubal (24.7828° N and 93.8859° E) and Bishnupur (23.0679° N and 87.3165° E).

Sampling Date: 1-15 of every alternate month from August 2014 - July 2017

Remarks: The revision of the genus *Paraquimperia* done by Moravec (1966, 1967) initially recognizes three species i.e., *P. anguillae, P. tenerrima* and *P. aditum* but after re-examining the co-types of *P. aditum*, it was found that *P. tenerrima* and *P. aditum* are conspecific. Moravec *et al*. (2000) described and added one new species, *P. Africana* from

Anguilla mossambica to the list. *Paraquimperia xenentodonia* described by Gupta and Bakshi (1984) from *Xenentodon cancila* is considered a species inquirenda (Naidu, 1983). Another species of *Paraquimperia* i.e., *P. manipurensis* has been first described from intestine of *Anabas testudineus* from Manipur (Shomorendra and Jha, 2003). Linstow (1878) described the nematode infecting *Anguilla anguilla* as *Nematoxys tenerrimus* which Baylis (1934) synonymized it to *Paraquimperia tenerrima*. Muller (1934) described the nematode from the same host as *Haplonema aditum* which is also a synonym of *P. tenerrima* (Moravec, 1966). Currently, there are four species of *Paraquimperia* including the nematode of our study viz, *P. tenerrima*, *P. africana, P. anguillae* and *P. manipurensis* (Shomorendra and Jha, 2003; Moravec and Scholz, 1991; Moravec *et al*., 2000).

Table 1.4 Morphometric measurements of *Paraquimperia manipurensis* collected from Manipur, India (N= 10)

Characters	Range (in mm)	Mean± Standard deviation
Body		
Length	4.00-9.05	6.52±2.52
Breadth	0.10-0.19	0.14±0.04
Eosophagus		
Length	1-1.40	1.20±0.2
Breadth	0.40-0.80	0.60±0.2
Cervical papillae	0.38-0.69	0.535±0.219
Vulva		
Length	0.03-0.04	0.03±0.01
Breadth	0.02-0.03	0.02±0.01
Eggs		
Length	0.08-0.10	0.090±0.014
Breadth	0.07-0.08	0.075±0.007
Spicules	0.07-0.12	0.095±0.035
Tail	0.17-0.59	0.380±0.296

Fig. 1.6 Light microscopic (Leica DM1000) images of *Paraquimperia manipurensis*

a) Curved anterior region of the body showing lateral alae (arrow)

b) Posterior third of female body with vulva (arrowhead) and eggs (arrow) inside the uterus

c) Tail end of the female showing anus (arrow)

d) Tail end of male

Subclass: Secernentea

Order: Spiruridea

Family: Camallanidae Railliet and Henry, 1915

Genus: *Procamallanus*

***Procamallanus* sp. Baylis, 1923**

(Fig. 1.7) (Table 1.5)

Total number of specimens collected: 9

Description: Body elongated and slender, buccal capsule present; capsule wall smooth, tridents absent; esophagus divided into anterior muscular corpus and longer posterior glandular part. Eosophagus clavate. Deirids present below and further away from buccal cavity on each side of lateral region. Tail conical, it's tip bluntly pointed, curved ventrad; caudal alae present, pairs of papillae, spicules well chitinized.

Host: *Anabas testudineus*

Habitat of the parasite: Intestine

Habitat of the host: Ponds, canals and lakes

Locality: Imphal East (24.7807° N and 93.9674° E), Imphal West (24.7828° N and 93.8859° E), Thoubal (24.7828° N and 93.8859° E) and Bishnupur (23.0679° N and 87.3165° E).

Sampling Date: 1-15 of every alternate month from August 2014 - July 2017

Remarks: The genus *Procamallanus* was erected by Baylis (1923) with *P. laeviconchus* as its type species. The key to the species of *Procamallanus* from fishes in South Asia was provided by Sood (1988). The genus *Procamallanus* consists of 34 species infecting different hosts (Yamaguti, 1961). Three new species *P. annulatus, P. monotaxis* (Moravec

and Justine, 2011) and *P. sinespinis* (Moravec and Justine, 2017) have been added to the list, where they are recovered from marine fishes of New Caledonia. *Procamallanus vysakhi*, another new species has been reported by Lakshmi (2010), recovered from the intestine of *Johnius carutta* from the Bay of Bengal at Visakhapatnam, India. The parasite of this genus has also been reported from Meghalaya by Jyrwa *et al*. (2016). From Manipur and Assam, the representative of this genus is reported as *P. saccobranchi* (Puinyabati *et al*., 2015). The collected nematode could not be identified up to the species level owing to unavailability of enough material for study purpose.

Table 1.5 Morphometric measurements of *Procamallanus* sp. collected from Manipur, India (N= 5)

Characters	Range (in mm)	Mean ± Standard deviation
Body		
Length	4.40-4.70	4.550±0.212
Breadth	0.24-0.25	0.420±0.007
Buccal cavity		
Length	0.08-0.09	0.085±0.007
Breadth	0.06-0.07	0.065±0.007
Anterior oesophagus	0.35-0.37	0.360±0.014
Posterior oesophagus	0.60-0.63	0.615±0.021
Sclerotized plate		
Length	0.07-0.08	0.245±0.007
Breadth	0.02-0.03	0.075±0.007

Spicule	0.41-0.43	0.025±0.014
Mucron	0.06-0.07	0.065±0.007

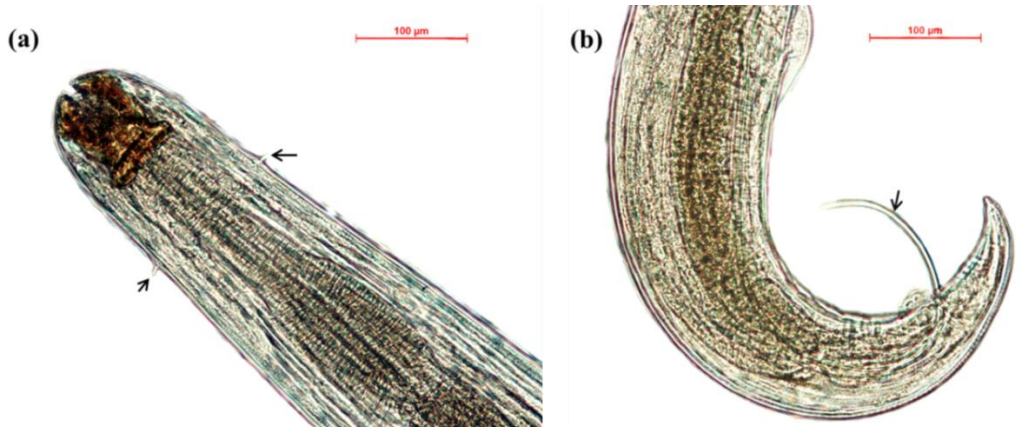

Fig 1.7 Light microscopic (Leica DM1000) images of *Procamallanus* sp.

a) Anterior end showing deirids (arrows)

b) Posterior end of male with spicule (arrow)

Order: Ascaridida

Family: Anisakidae Skrjabin & Karkokhin, 1945

Genus: *Anisakis* Dujardin, 1845

***Anisakis* sp. Dujardin, 1845**

(Fig. 1.8) (Table 1.6)

Total number of specimens collected: 25

Description: Anterior part of the body with prominent stylet-like structure, a pair of boring tooth; the larval forms are found ensheathed by a very hard protective layer. Labia and labial lips inconspicuous. Anterior oesophagus is muscular and a glandular part is ventriculus. Body cuticle striated; the tail end is sharply curved and has a pointed tip.

Host: *Channa gachua*

Habitat of the parasite: Body cavity and outer lining of intestines

Habitat of the host: Canals, lakes and ponds.

Locality: Thoubal (24.7828° N and 93.8859° E), Bishnupur (23.0679° N and 87.3165° E) and Ukhrul (25.0954° N and N 94.3617° E).

Sampling Date: 1-15 of every alternate month from August 2014 - July 2017

Remarks: The genus *Anisakis* was established by Dujardin, 1845. The nematodes of this genus are the most important among the the family Anisakidae because of the pathogenicity of its L3 larvae (Bilska-Zajac *et al*., 2015). These nematodes are common parasites of marine fish and potential risk for human health (Cruz *et al*., 2009). This parasite has also been reported from Tripura by Koiri and Roy (2016). The present study is the first report of the occurrence of *Anisakis* sp. larvae from the freshwater fish *Channa gachua* of Manipur, India.

Table 1.6 Morphometric measurements of *Anisakis* sp. collected from Manipur, India (N= 5)

Characters	Range (in mm)	Mean±Standard deviation
Body Length	5.40-5.60	5.500±0.141
Body Breadth	0.24-0.27	0.255±0.021
oesophagus	1.30-1.37	1.335±0.049
Glandular ventricles	1.50-1.55	1.525±0.035
Mucron	0.06-0.07	0.065±0.007

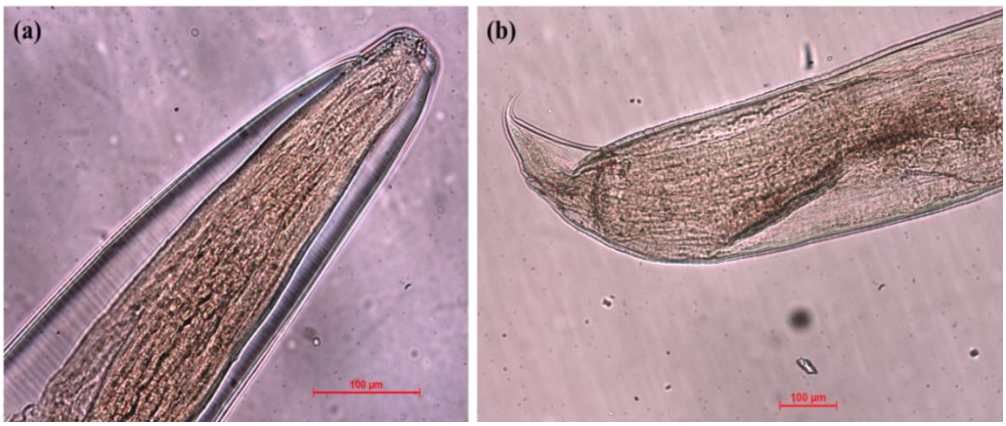

Fig. 1.8 Light microscopic (Leica DM1000) images of *Anisakis* sp.

a) Anterior end

b) Posterior end

Class: Cestoda

Order: Caryophyllidea Wardle & McLeod, 1952

Family: Lytocestidae Hunter 1927

Genus: *Lytocestus* Cohn, 1908

Species: *L. attenuatus* Tandon *et al*., 2005

(Fig. 1.9) (Table 1.7)

Total number of specimens collected: 251

Description: Body slender, elongated, posterior end broader than anterior end. Scolex without hooks, rounded end and not differentiated; neck fairly long. Testes medullary and

ovoid in shape; vitelline follicles ovoid, placed in two rows lateral to testes up to cirrus sac; cirrus sac medullary. Ovary bilobed, inverted A-shaped, follicular, lobes extending to posterior level to Mehlis' gland and joined each other by ovarian isthmus; ovarian lobes cortical, isthmus medullary. Uterus glandular and extends behind Mehlis's gland; excretory pore terminal.

Host: *Clarias magur*

Habitat of the parasite: Intestine

Habitat of the host: Ponds and lakes

Locality: Imphal East (24.7807° N and 93.9674° E), Imphal West (24.7828° N and 93.8859° E), Thoubal (24.7828° N and 93.8859° E) and Bishnupur (23.0679° N and 87.3165° E).

Sampling Date: 1-15 of every alternate month from August 2014 - July 2017.

Remarks: The species *L. attenuates* was established by Tandon *et al*., (2005) from *Clarias magur* of Guwahati, Assam and Shella, Meghalaya. This species was compared to Indo-Malaysian species like *L. javanicus, L. parvulus, L. longicollis, L. filiformis* and *L. lativitellarium* and it showed similarity in some morphological features

and not completely alike with any of the mentioned species. The major differences from all other forms are having genital atrium and spinous egg. This species has been reported from Tripura by Koiri and Roy (2017b) and Manipur by Mangolsana *et al*. (2016).

Table 1.7 Morphometric measurements of *Lytocestus attenuatus* collected from Manipur, India (N= 10)

Characters	Range (in mm)	Mean± Standard deviation
Body		

Length	11.0-13.0	12.000±1.414
Breadth	0.60-0.70	0.650±0.070
Scolex length	0.14-0.15	0.145±0.007
Neck length	5.00-6.00	5.500±0.707
Testicular follicles		
Length	0.06-0.08	0.070±0.014
Breadth	0.03-0.04	0.035±0.007
Ovarian lobes		
Length	0.04-0.06	0.050±0.014
Breadth	0.03-0.05	0.040±0.014
Vitelline follicles		
Length	0.05-0.07	0.060±0.014
Breadth	0.02-0.03	0.025±0.007

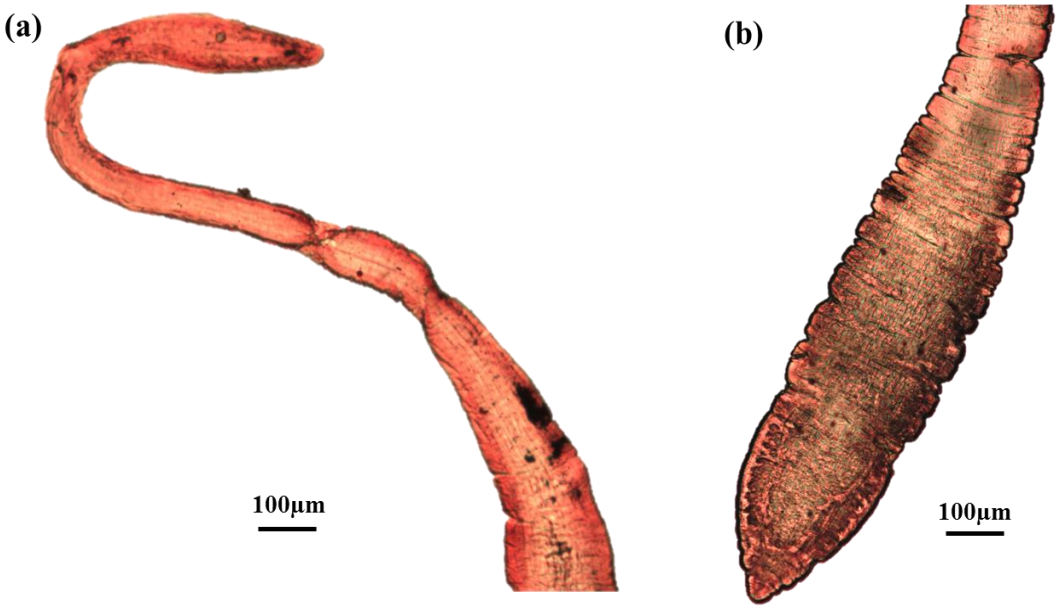

Fig. 1.9 Light microscopic (Leica DM1000) images of *Lytocestus attenuatus* (stained in Borax carmine)

a) Anterior half of the body showing slender and elongated body

b) Posterior part of the body showing cuticle with pseudo-segment like division

Genus: *Lytocestus* Cohn, 1908

Species: *L. indicus* (Moghe, 1925) Woodland, 1926

(Fig. 1.10) (Table 1.8)

Total number of specimens collected: 522

Description: Body large, broadest at the level of cirrus sac; scolex rounded, unarmed, and stout. Body divided into outer and inner layers of longitudinal muscles. Neck short, narrow and undifferentiated. Testes numerous occupying medullary region of the body, oval in shape, larger than vitelline follicles and extends from the base of the neck to the cirrus sac region posteriorly. Ovary bilobed, joined to each other by ovarian isthmus, wing-like shaped, follicular; Mehli's gland situated behind ovarian isthmus; vitelline follicles corticular, in a ring around the testes, no post ovarian vitelline follicles present. Eggs ovoid, smooth, embryonated.

Host: *Clarias magur*

Habitat of the parasite: Intestine

Habitat of the host: Ponds and lakes

Locality: Imphal East (24.7807° N and 93.9674° E), Imphal West (24.7828° N and 93.8859° E), Thoubal (24.7828° N and 93.8859° E) and Bishnupur (23.0679° N and 87.3165° E).

Sampling Date: 1-15 of every alternate month from August 2014 - July 2017.

Remarks: The genus *Lytocestus* was created by Cohn (1908) with *L. adhaerens* as type species from *Clarias fuscus*, from Hongkong. There are several reports on this genus from India. *L. indicus* was first described by Moghe (1925) from *Clarias magur*, siluriod fish from India and was placed under the genus *Caryophyllaeus*. But Woodland (1926) moved it to the genus *Lytocestus* clarifying that the post-ovarian follicles described were actually

ovarian follicles. The same species was later reported from *Clarias magur* and *Clarias magur* in India by Mehra (1930). This species has also been reported from other states of the Northeast India. Chakravarty and Tandon (1988) reported the species from Guwahati, Assam; Koiri and Roy (2017b), recorded from Tripura; Devi *et al*. (2017b), reported from Manipur.

Table 1.8 Morphometric measurements of *Lytocestus indicus* collected from Manipur, India (N= 10)

Characters	Range (in mm)	Mean± Standard deviation
Body		
Length	4.00-7.00	5.500±2.121
Breadth	1.50-2.00	0.060±0.042
Scolex length	0.35-0.50	0.42±50.353
Neck length	0.50-0.55	0.525±0.106
Testicular follicles		
Length	0.04-0.10	1.750±0.035
Breadth	0.03-0.09	0.070±0.042
Ovarian lobes		
Length	0.02-0.02	0.020±0.00
Breadth	0.02-0.03	0.025±0.007
Vitelline follicles		
Length	0.02-0.04	0.030±0.014
Breadth	0.02-0.03	0.02±50.007

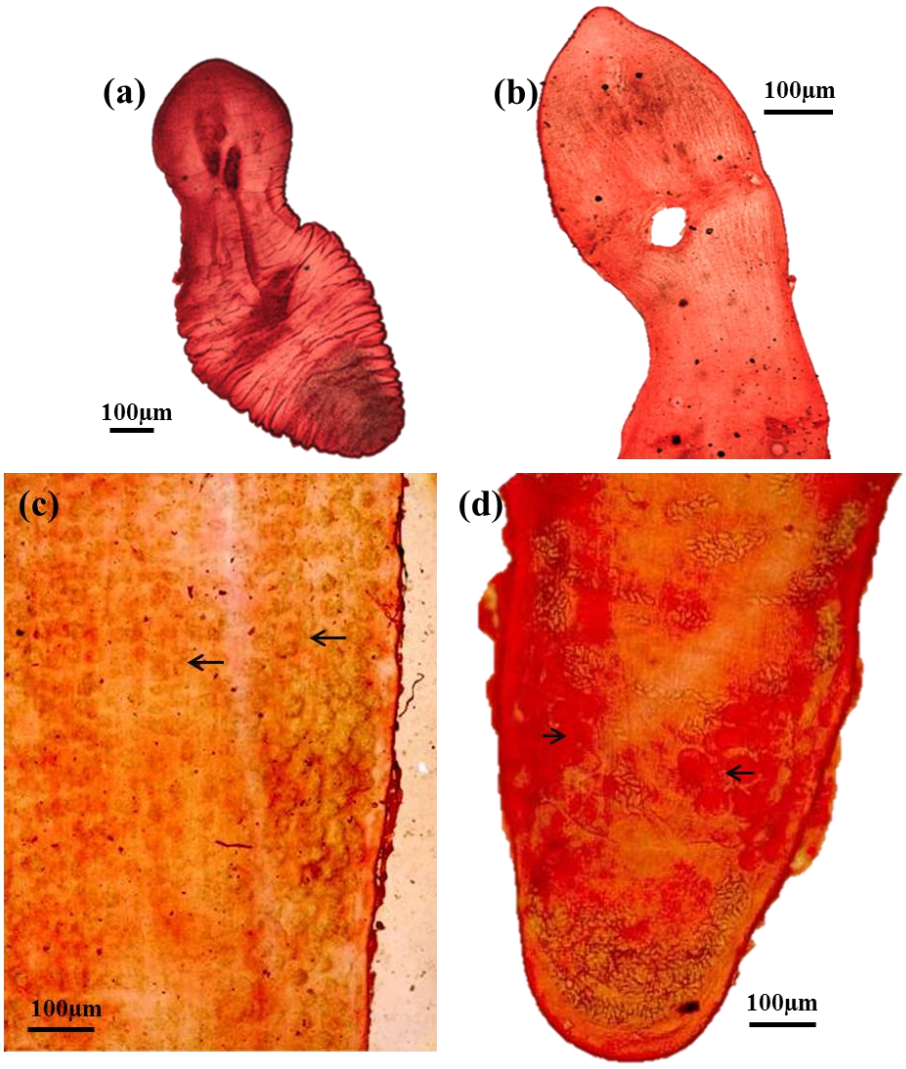

Fig. 1.10 Light microscopic (Leica DM1000) images of *Lytocestus indicus* (stained in Borax carmine)

a) Larval form of the worm

b) Anterior region showing rounded scolex with undifferentiated neck

c) Middle region of the body with numerous testes (arrows)

d) Posterior region with wing-shaped biloped ovary (arrows)

Genus: *Lytocestus* Cohn, 1908

Species: *L. filiformes* (Woodland, 1923) Fuhrmann and Baer, 1925

(Fig. 1.11) (Table 1.9)

Total number of specimens collected: 320

Description: Body long, filiform, posterior end broader than the anterior end, maximum breadth at level of cirrus sac; longitudinal muscle fibers inclined in two distinct regions of cortex and medulla. Scolex unarmed, undifferentiated and flat in shape. Neck elongated and slender. Testes present in the medullary region of the body, numerous in numbers, spherical or oval in shape extending from behind the neck up to the cirrus sac posteriorly; cirrus bordered by a thin muscular wall, opening separately from the utero-vaginal pore. Ovary bilobed, follicular, cortical, the two lobes joined with each other by an ovarian isthmus; Mehlis' gland well developed behind ovarian isthmus; uterine coils extends behind the isthmus till the anterior part of ovary and cirrus sac; vagina distinct, joins the uterus distally to open at the utero-vaginal pore. Vitellaria cortical, smaller than testes, spherical or oval in shape, form a crescent around the testes, no post-ovarian vitelline follicles present. Excretory pore terminal. Eggs smooth and oval in shape.

Host: *Clarias magur*

Habitat of the parasite: Intestine

Habitat of the host: Ponds and lakes

Locality: Imphal East (24.7807° N and 93.9674° E), Imphal West (24.7828° N and 93.8859° E), Thoubal (24.7828° N and 93.8859° E) and Bishnupur (23.0679° N and 87.3165° E).

Sampling Date: 1-15 of every alternate month from August 2014 - July 2017.

Remarks: *Lytocestus filiformis* was first described as *Caryophyllaeus filiformis* by Woodland (1923) from *Mormyrus caschive* of Egypt Sudan. It was later placed under the genus *Lytocestus* by Fuhrmann and Baer (1925) based on cortical disposition of vitellaria and medullary disposition of testes. This species has been reported from Assam, Meghalaya and Manipur in Northeast India (Jyrwa *et al*., 2016; Devi *et al*., 2017).

Table 1.9 Morphometric measurements of *Lytocestus filiformes* collected from Manipur, India (N= 10)

Characters	Range (in mm)	Mean± Standard deviation
Body		
Length	15.00-19.00	17.00±2.828
Breadth	0.50-0.60	0.550±0.070
Neck length	5.0-5.23	5.115±0.162
Testicular follicles		
Length	0.04-0.06	0.050±0.014
Breadth	0.12-0.16	0.140±0.028
Ovarian lobes		
Length	0.03-0.04	0.035±0.007

Breadth	0.04-0.05	0.045±0.007
Vitelline follicles		
Length	0.05-0.07	0.060±0.014
Breadth	0.02-0.03	0.025±0.007
Eggs		
Length	0.03-0.04	0.035±0.007
Breadth	0.02-0.03	0.025±0.007

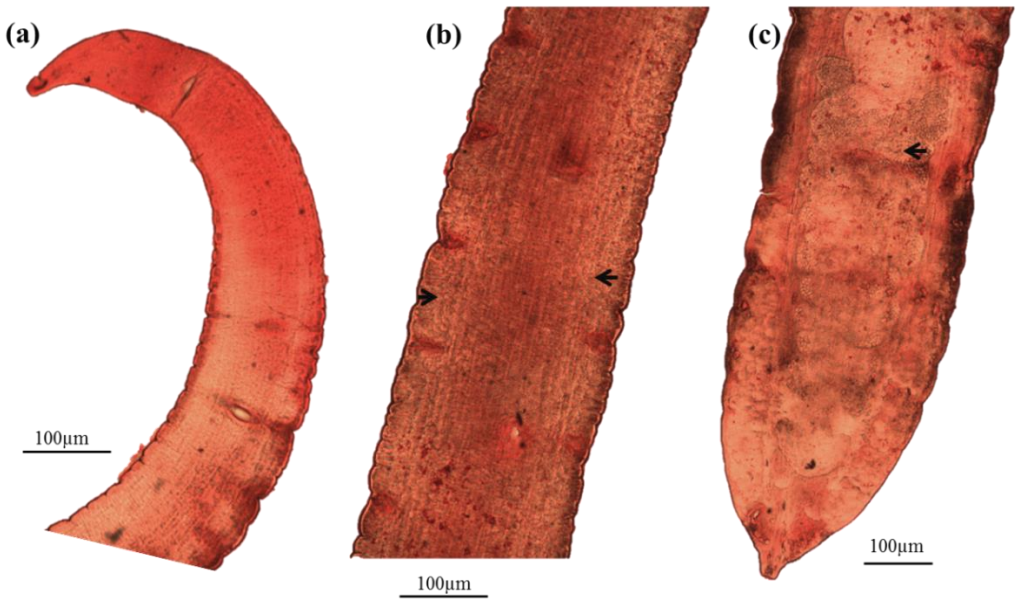

Fig. 1.11 Light microscopic (Leica DM1000) images of *Lytocestus filiformis* (stained in Borax carmine)

a) Anterior region showing filiform scolex and broad neck

b) Mid-body with vitellaria (arrows)

c) Posterior end of the body showing cirrus sac (arrow) and various reproductive organs

Genus: *Lytocestus* Cohn, 1908

Species: *L. longicollis* Rama Devi, 1973

(Fig. 1.12) (Table 1.10)

Total number of specimens collected: 254

Description: Scolex tumid, spatulate or oblong, undifferentiated and unarmed; body proper short; neck exceedingly long, occupying one third of the body length; body proper divided into an outer cortex and an inner medulla by two distinct layers of parenchymal longitudinal muscles; testes multitudinous and occupies the posterior region of the body, extends from anterior end of the body to cirrus sac posteriorly, spherical in shape; cirrus sac ovoid, bound by thin muscular wall enclosing the long ductus ejaculatorious that opens separately from the utero-vaginal pore. Ovary H-shaped, bilobed, follicular, connected by ovarian isthmus and corticular in depositon. Mehlis' gland situated posterior to isthmus in

between the two ovarian lobes. Vitellaria cortical, smaller than testes, extending from a few millimeters anterior to the testes up to the cirrus sac, no post-ovarian vitelline follicles present. Excretory pore terminal.

Host: *Clarias magur*

Habitat of the parasite: Intestine

Habitat of the host: Ponds and lakes

Locality: Imphal East (24.7807° N and 93.9674° E), Imphal West (24.7828° N and 93.8859° E), Thoubal (24.7828° N and 93.8859° E) and Bishnupur (23.0679° N and 87.3165° E).

Sampling Date: 1-15 of every alternate month from August 2014 - July 2017.

Remarks: Rama Devi (1973) described *L. longicollis* for the first time from *Clarias mmagur* in Vishakapatnam, Andra Pradesh. This species has exceptionally long neck, thus named *longicollis*. It is also different from other lytocestid in having receptaculum eminis. Occurrence of the same species has also been reported from Godavari river by Vankara *et al*. (2014). In Northeast India, it was first reported by Chakravarty and Tandon (1988) from Guwahati (Assam). Koiri and Roy (2016) have reported the occurrence of the parasite from Tripura. This species has also been reported from Manipur by Devi *et al*. (2017).

Table 1.10 Morphometric measurements of *Lytocestus longicollis* collected from Manipur, India (N= 10)

Characters	Range (in mm)	Mean± Standard deviation
Body		
Length	10.00-16.00	13.00±4.242
Breadth	0.40-0.45	0.425±0.035

Neck length	7.60-8.00	7.800±0.282
Testicular follicles		
Length	0.05-0.06	0.055±0.007
Breadth	0.06-0.07	0.065±0.007
Ovarian lobes		
Length	0.03-0.04	0.035±0.007
Breadth	0.04-0.05	0.045±0.007
Vitelline follicles		
Length	0.03-0.05	0.040±0.014
Breadth	0.02-0.04	0.030±0.014

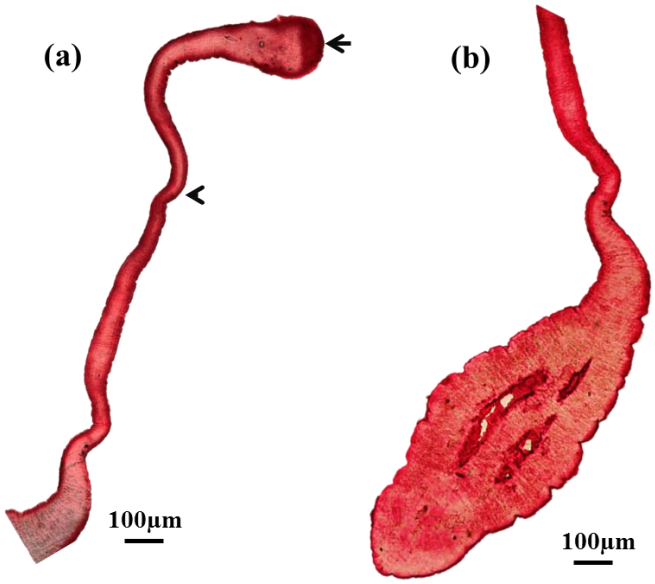

Fig 1.12 Light microscopic (Leica DM1000) images of *Lytocestus longicollis* (stained in Borax carmine)

a) Anterior region of the body showing tumid scolex (arrow) and very long neck (arrowhead)

b) Posterior end of the body

Genus: *Djombangia* Bovien, 1926

Species: *Djombangia penetrans* Bovien, 1926

(Fig. 1.13) (Table 1.11)

Total number of specimens collected: 277

Description: Scolex globate with a terminal sucker; Neck well set off from the body. Body short, broad, maximum breadth at the level of cirrus sac; body divided into an outer cortex and an inner medulla by two layers of longitudinal muscles. Testes numerous, spherical or ovoid, extending in two lateral rows from outstart of body proper up to the level just in front of the ovary; cirrus pouch not well defined, opening into a common atrium immediately in front of the utero-vaginal pore; genital atrium close to posterior extremity, just in front of the ovarian isthmus. Ovary bilobed, at posterior extremity, follicular, the two lobes connected to each other by an ovarian isthmus; uterus partly glandular, its coils largely in the median field of medulla, and reaching up to the commencement of testicular region. Vitellaria globular, extending in the cortical parenchyma of testicular and ovarian zone; no post-ovarian vitelline follicles present. Eggs prolate and operculate.

Host: *Clarias magur*

Habitat of the parasite: Intestine

Habitat of the host: Ponds and lakes

Locality: Imphal East (24.7807° N and 93.9674° E), Imphal West (24.7828° N and 93.8859° E), Thoubal (24.7828° N and 93.8859° E) and Bishnupur (23.0679° N and 87.3165° E).

Sampling Date: 1-15 of every alternate month from August 2014 - July 2017.

Remarks: The genus *Djombangia* was erected by Bovien (1926) with *D. penetrans* as type specimen from *Clarias magur* in Java. Yamaguti (1959) recognized *D. penetrans* to be the only species under the genus *Djombangia*. Few more species were added under this genus, but in India the genus *Djombangia* is represented by *D. penetrans* and *D. indica* (Mackiewicz, 1981). *D. penetrans* has been reported from other Northeast states of India, viz., Assam, Mehgalaya, Tripura and Manipur (Charavarty and Tandon, 1988; Jyrwa, 2016; Koiri and Roy, 2016; Devi *et al*., 2017).

Table 1.11 Morphometric measurements of *Djombangia penetrans* collected from Manipur, India (N= 10)

Characters	Range (in mm)	Mean± Standard deviation
Body		
Length	6.72-11.00	8.860±3.026
Breadth	2.23-4.21	3.220±1.400
Neck		
Length	0.70-0.82	0.760±0.084
Breadth	0.60-0.78	0.690±0.127
Testicular follicles		
Length	0.09-0.10	0.095±0.007
Breadth	0.04-0.06	0.050±0.014
Ovarian lobes		
Length	0.31-0.76	0.535±0.318
Breadth	0.97-1.33	1.150±0.254
Vitelline follicles		
Length	0.03-0.04	0.035±0.007
Breadth	0.02-0.03	0.025±0.007
Eggs (a)Length	0.05-0.06	0.055±0.007
Breadth	0.03-0.04	0.035±0.007

Distance between anterior extent of testes and vitellaria	0.55-0.69	0.620±0.098
Position of the genital pore from the posterior extremity	0.93-1.50	1.215±0.403

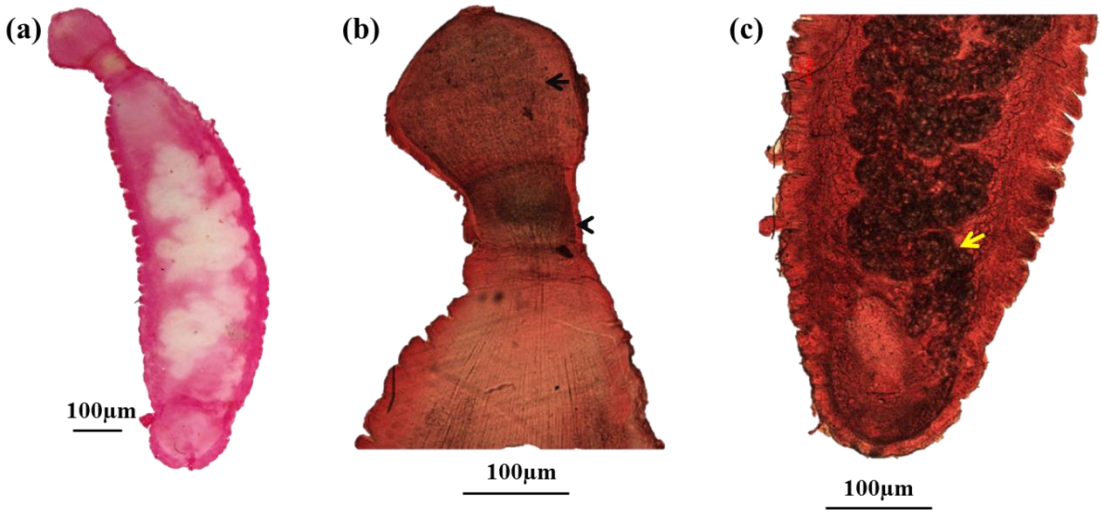

Fig. 1.13 Light microscopic (Leica DM1000) images of *Djombangia penetrans* (stained in Borax carmine)

a) General view of whole body

b) Anterior region showing rounded scolex (arrow) and neck (arrowhead)

c) Posterior end of the body showing uterus filled with eggs (arrow)

Order: Pseudophyllidea Carus, 1863

Family: Bothriocephalidae Blanchard, 1849

Genus: *Senga* Dollfus, 1934

Species: *S. lucknowensis* Johri, 1956

(Fig. 1.14) (Table 1.12)

Total number of specimens collected: 15

Description: Bothria is rectangular; apical disc present. Body long and segmented; proglottids acraspedote and wide, but gravid portion intended rather than segmented and long; neck absent; Testes numerous, small, rounded and located in two lateral fields and not compact; ovary flattened and compact, transversely elongated; genital pore dorsal, virtually median; vitelline follicles cortical; the gravid proglottid contains fertilized egg dispersed in the posterior region of the strobili; unoperculated eggs and not embryonated.

Host: *Channa punctata*

Habitat of the parasite: Intestine

Habitat of the host: Loktak Lake

Locality: Bishnupur (23.0679° N and 87.3165° E).

Sampling Date: 25th March, 2015.

Remarks: Dollfus, 1934 established the genus *Senga* with *Senga besnardi* as type specimen parasitizing *Betta splendens* from Vincennes, France. Johri (1956) described *S. lucknowensis* from *Mastacembellus armatus* from India. Many species of *Senga* have been reported from India {(*S. satarensis* from Maharashtra Bhure and Nanware (2011); *S. Rupchandensis* from Maharashtra Pardeshi and Hiware (2011); *S. govindii* from Maharashtra Jadhav *et al*. (2012); *Senga jadhavii* from Godavari basin, Fartade and Fartade (2015); *S. rostellata* from Maharashtra Deshmukh *et al*. (2016)}. Occurrence of *Senga* has been reported from Northeast India, however identification up to species level were not provided. In the present study, it is identified up to the species level as *S. lucknowensis* and this is the first report of the occurrence of *S. lucknowensis* from Manipur, Northeast India.

Table 1.12 Morphometric measurements of *Senga lucknowensis* collected from Manipur, India (N= 5)

Characters	Range (in mm)	Mean± Standard deviation
Body		
Length	3.45-22.00	12.725±13.116
Breadth	0.42-0.47	0.445±0.035
Bothria		
Length	0.65-0.69	0.670±0.028
Breadth	0.34-0.36	0.350±0.014
Immature segments		
Length	0.32-0.35	0.335±0.021

Breadth	0.45-0.51	0.480±0.030
Mature segments		
Length	0.15-0.19	0.170±0.028
Breadth	0.21-0.23	0.22±0.010

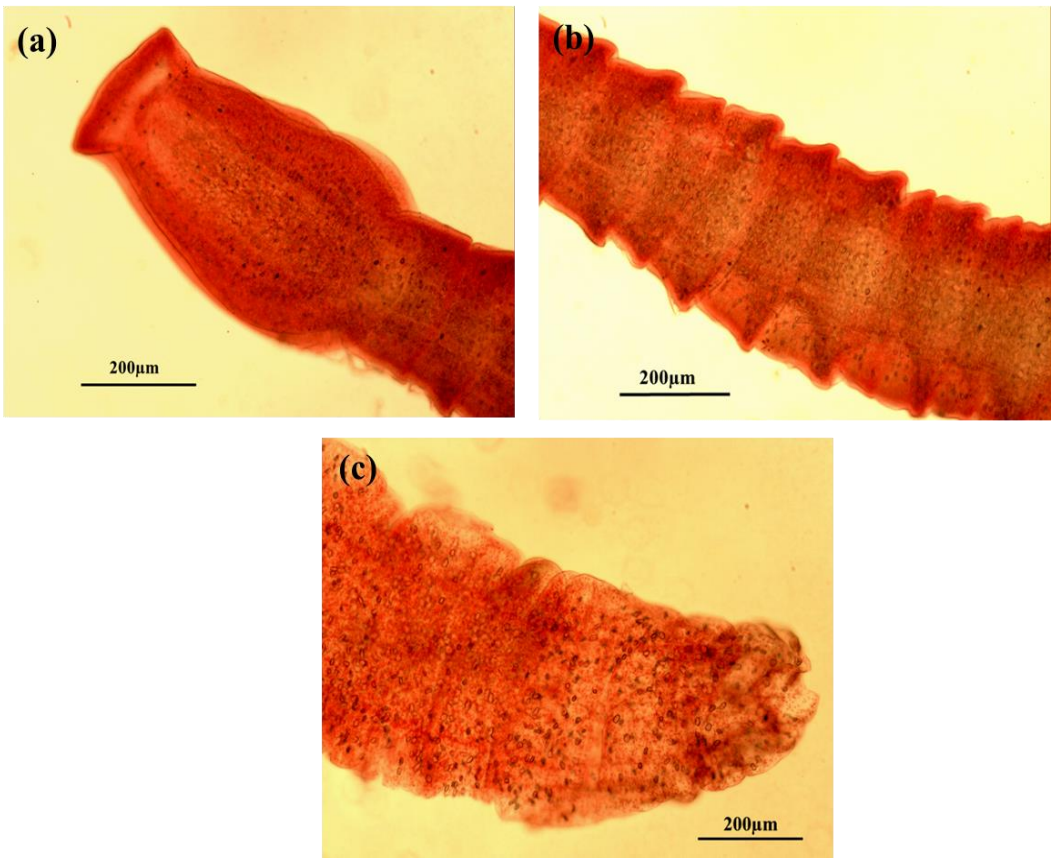

Fig. 1.14 Light microscopic (Leica DM1000) images of *Senga lucknowensis* (stained in Borax carmine)

a) Anterior region of the body showing scolex with rectangular bothria

b) Mid-region of the body showing body metamerism

c) Posterior end of the body showing the presence of eggs

Class: Trematoda Rudolphi, 1808

Order: Plagiorchiida La Rue, 1957

Family: Plagiorchiidae Lühe, 1901

Genus: *Astiotrema* Looss, 1900

Species*: A. reniferum* (Looss, 1998) Stossich, 1904

(Fig. 1.15) (Table 1.13)

Total number of specimens collected: 2

Description: Body lanceolate with rounded apex; tegument armed with spines where the spines are directed backwards; oral sucker sub-terminal; ventral sucker is almost equal to the size of oral sucker and located at one third of the body length, to the right of ventral sucker is the curved cirrus sac; genital pore present immediately above the acetabulum; Intestine bifurcate in front of the ventral sucker; intestinal caeca broad to narrow, extends

posteriorly; vitellaria arranged at the lateral sides of the body; testes rounded with tandem arrangement in posterior half of the body.

Host: *Clarias magur*

Habitat of the parasite: Intestine

Habitat of the host: Pond

Locality: Imphal West (24.7828° N and 93.8859° E)

Sampling Date: 5th May, 2016

Remarks: *Astiotrema reniferum* was first established as *Distoma reniferum* by Looss and later renamed it as *Astiotrema reniferum* (Siddiqi, 1965). Review done by Yeh and Fotedar (1958) recognized four species (*A. reniferum*; *A. impletum*; *A. odhneri* and *A. monticelli*) to be valid out of 21 species described. Occurrence of *A. reniferum* has been reported from Tripura and Manipur (Puinyapati *et al*., 2010; Koiri and Roy, 2017b).The parasite is regarded as a rare species infecting Clarid fishes (Zhokhov *et al*., 2017)

Table 1.13 Morphometric measurements of *Astiotrema reniferum* collected from Manipur, India (N= 2)

Characters	Range (in mm)	Mean± Standard deviation
Body		
Length	1.85-1.87	1.860±0.014
Breadth	0.35-0.36	0.355±0.007
Oral sucker		
Length	0.01-0.01	0.010±0.00
Breadth	0.01-0.02	0.015±0.007
Pre-pharynx		
Length	0.05-0.06	0.055±0.007

Breadth	0.03-0.04	0.035±0.007
Pharynx		
Length	0.06-0.07	0.065±0.007
Breadth	0.04-0.05	0.045±0.007
Oesophagus		
Length	0.26-0.27	0.265±0.007
Breadth	0.04-0.05	0.045±0.007
Acetabulum		
Length	0.15-0.16	0.155±0.007
Breadth	0.18-0.19	0.185±0.007
Anterior testes		
Length	0.16-0.17	0.165±0.007
Breadth	0.15-0.16	0.155±0.007
Posterior testes		
Length	0.22-0.23	0.225±0.007
Breadth	0.15-0.16	0.155±0.007
Cirrus sac		
Length	0.20-0.21	0.205±0.007
Breadth	0.05-0.06	0.055±0.007
Ovary		
Length	0.16-0.17	0.165±0.007
Breadth	0.13-0.14	0.135±0.007
Eggs		
Length	0.03-0.04	0.035±0.007
Breadth	0.02-0.03	0.025±0.007

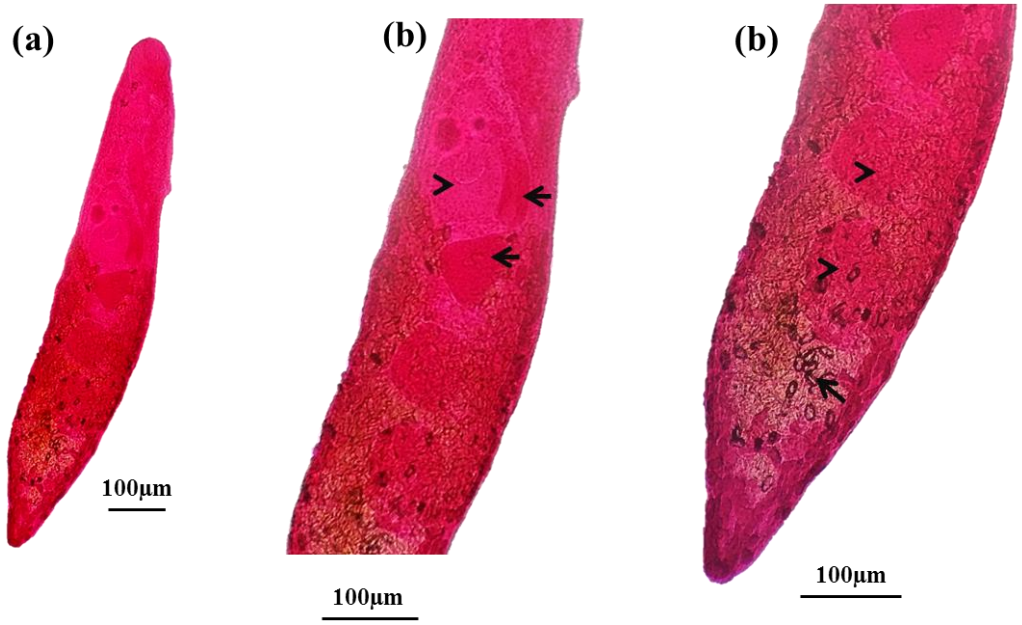

Fig. 1.15 Light microscopic (Leica DM1000) images of *Astiotrema reniferum* (stained in Borax carmine)

a) General view of the whole worm

b) Mid-region showing acetabulum (arrowhead), cirrus sac and ovary (arrows)

c) Posterior end of the body showing the presence of egg in uterus (arrow) and testes (arrowheads)

Order: Strigeatida La Rue, 1926

Family: Clinostomadae Luhe, 1901

Genus: *Clinostomum* Leidy, 1856

Species: *C. philippinense* Velasquez, 1960

(Fig. 1.16) (Table 1.14)

Total number of specimens collected: 7

Description: Body medium, linguiform, dorsally convex, oral sucker is smaller than the ventral sucker and surrounded by a collar like structure; anterior and posterior end rounded, esophagus bifurcates posterior to oral sucker into two intestinal caeca and continues till the terminal end of the body. Ovary is intertesticular and submedian.

Host: *Trichogaster fasciata*

Habitat of the parasite: Intestine

Habitat of the host: Canal

Locality: Lamphelpat- Imphal West (24.8236° N, 93.9114° E).

Sampling Date: 7th January, 2016

Remarks: The genus *Clinostomum* was created by Leidy (1856) to accommodate *Diplostomum complanatum* Rudolphi (1819). The life cycle of *Clinostomum* involve gastropods as the first intermediate host, fishes or amphibians as second intermediate host and birds as the definitive host. They are found to infect human beings occasionally. Many metacercariae have been reported from various fishes all over the world (Tiewchaloern *et al*., 1999; Park *et al*., 2009; Yooyen *et al*., 2006; Gustinelli *et al*., 2010; Wang *et al*., 2017; Caffara *et al*., 2017). In India, the *Clinostomum* species of common occurrence is

represented by *C. complanatum* (Shareef and Abidi, 2012; Rizvi *et al*., 2012). The present study is the first report of the occurrence of *C. philippinense* from India.

Table 1.14 Morphometric measurements of *Clinostomum philippinense* collected from Manipur, India (N= 5)

Characters	Range (in mm)	Mean± Standard deviation
Body		
Length	2.35-5.54	3.945±2.255
Breadth	1.60-2.19	1.895±0.417
Oral sucker	0.25-0.35	0.300±0.070
Ventral sucker		
Length	0.83-1.15	0.990±0.226
Breadth	0.81-1.14	0.975±0.233
Anterior testes		
Length	0.30-0.54	0.420±0.169
Breadth	0.32-0.51	0.415±0.134
Posterior testes		
Length	0.19-0.49	0.340±0.212
Breadth	0.31-0.71	0.510±0.282

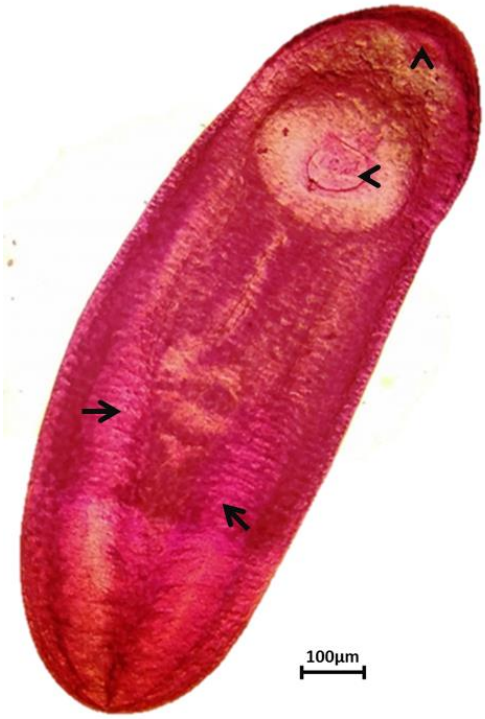

Fig. 1.16 Light microscopic (Leica DM1000) image of *Clinostomum philippinense* (stained in Borax carmine)

General view of the whole worm with prominent suckers (arrowheads) and intestinal caecum (arrows)

Family: Diplostomadae Poirier, 1886

Genus: *Posthodiplostomum* Dubois, 1936

***Posthodiplostomum* sp.**

(Fig. 1.17) (Table 1.15)

Total number of specimens collected: 30

Description: Excysted metacercaria with distinct division of body into two parts; forebody elongated, suckers weakly developed, intestinal caeca not prominent; holdfast gland present. Hind body short cylindrical to oval; testes two, lying one next to another; anterior testis almost the same size as posterior testis, oval, sub-medially placed; transversely elongate; ovary small, rounded and sub-median.

Host: *Channa punctata* and *Channa gachua*

Habitat of the parasite: Intestine and muscle

Habitat of the host: Ponds

Locality: Imphal West (24.7828° N and 93.8859° E), Thoubal (24.7828° N and 93.8859° E) and Bishnupur (23.0679° N and 87.3165° E).

Sampling Date: August, 2016

Remarks: *Posthodiplostomum* was established by Dubois (1936) with *P. cuticola* as the type species from avian hosts. Later, *P. brevicaudatum* and *P. minimum* were also reported

from fish hosts (Yamaguti, 1971). Since then, the parasite of this genus has been reported from different fishes by several workers (Lane and Morris, 2000; Ondrackova *et al*., 2004; Kvach *et al*., 2017; Stoyanov, 2017). *P. cuticola, P. grayii P. milvi* and *P. mehtai* have been reported from India (Yamaguti, 1971). *Posthodiplostomum sp*. was reported for the first time from Manipur, Northeast India, by Athokpam and Tandon (2014).

Table 1.15 Morphometric measurements of *Posthodiplostomum sp.* collected from Manipur, India (N= 5)

Characters	Range (in mm)	Mean±Standard Deviation
Body		
Length	1.52-1.55	1.535±0.021
Breadth	0.36-0.38	0.370±0.014
Oral sucker		
Length	0.01–0.03 0.02–0.03	0.020±0.014
Breadth		0.025±0.007
Acetabulum		
Length	0.07-0.08 0.05-0.06	0.075±0.007
Breadth		0.055±0.007
Holdfast		
Length	0.20-0.22	0.210±0.014
Breadth	0.15-0.16	0.155±0.007
Anterior testes		
Length	0.10-0.12	0.110±0.014

Breadth	0.14-0.16	0.150±0.014
Posterior testes		
Length	0.09-0.10	0.095±0.007
Breadth	0.13-0.14	0.135±0.007

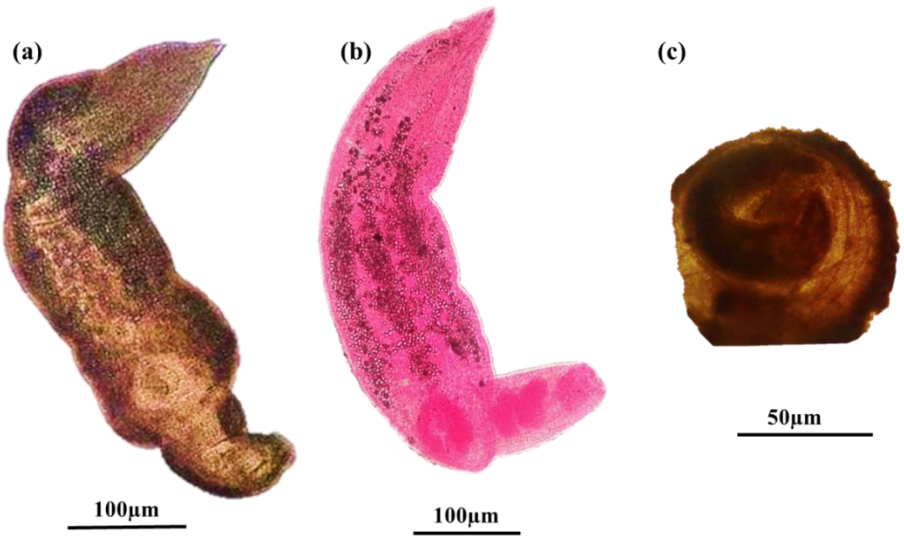

Fig. 1.17 Light microscopic (Leica DM1000) images of *Posthodiplostomum* sp. (stained in Borax carmine)

a) & (b) General view of excysted metacercaria

c) Encysted metacercaria

Order: Plagiorchiida

Family: Gorgoderidae Looss, 1899

Genus: *Phyllodistomum* Braun, 1899

***Phyllodistomum* sp.**

(Fig. 1.18) (Table 1.16)

Total number of specimens collected: 10

Description: Body spatulated, hind body broad and cordate shaped, forebody relatively very short, tapering gradually towards the anterior end. Oral sucker slightly bigger than the acetabulum and acetabulum situated slightly in front of the mid-body; pre-pharynx and pharynx absent, oesophagus short and stout, intestinal bifurcation pre-acetalur and caeca extends near posterior end of the body. Testes two, lobed, oblique and present in the broadest part of the body; ovary rounded to weakly lobed. Two lobed vitelline glands

situated close to one another below the acetabulum. Uterus highly coiled and profusely distributed all over in the hind body.

Host: *Channa gachua*

Habitat of the parasite: Intestine

Habitat of the host: Ponds

Locality: Bishnupur (23.0679° N and 87.3165° E).

Sampling Date: 10th August, 2016

Remarks: *Phyllodistomum* was created by Braun (1899) with *Distomum folium* as type species. Many species of *Phyllodistomum* have been described since then. This genus is believed to contain more that 110 species (Zhokov and Zoxob, 2010). However, numerous synonymy are observed, the factor being lack of knowledge of the entire group and changing concept and diagnosis of the genus *Catoptroides* (Lewis, 1935; Pérez-Ponce de león *et al*., 2015). The specimen shows a great resemblance to *Phyllodistomum sp.* in all morphological traits. This is the first report for the occurrence of the genus *Phyllodistomum* from Manipur, Northeast India.

Table 1.16 Morphometric measurements of *Phyllodistomum* sp. collected from Manipur, India (N= 4)

Characters	Range (in mm)	Mean ± Standard deviation
Body		
Length	1.65-2.7	2.175 ± 0.742
Breadth	1.60-2.40	2.000 ± 0.565
Oral sucker		
Length	0.40-0.47	0.435 ± 0.049

Breadth	0.43-0.50	0.465 ± 0.049
Acetabulum		
Length	0.37-0.44	0.405 ± 0.049
Breadth	0.40-0.46	0.430 ± 0.042
Anterior end to acetabulum	0.63-0.80	0.715 ± 0.120
Anterior testes		
Length	0.16-0.25	0.205 ± 0.063
Breadth	0.10-0.21	0.155 ± 0.077
Posterior testes		
Length	0.17-0.30	0.235 ± 0.091
Breadth	0.21-0.31	0.260 ± 0.070
Vitelline gland		
Length	0.20-0.26	0.230 ± 0.042
Breadth	0.17-0.20	0.18 5± 0.021
Ovary		
Length	0.30-0.32	0.310 ± 0.014
Breadth	0.36-0.39	0.375 ± 0.021

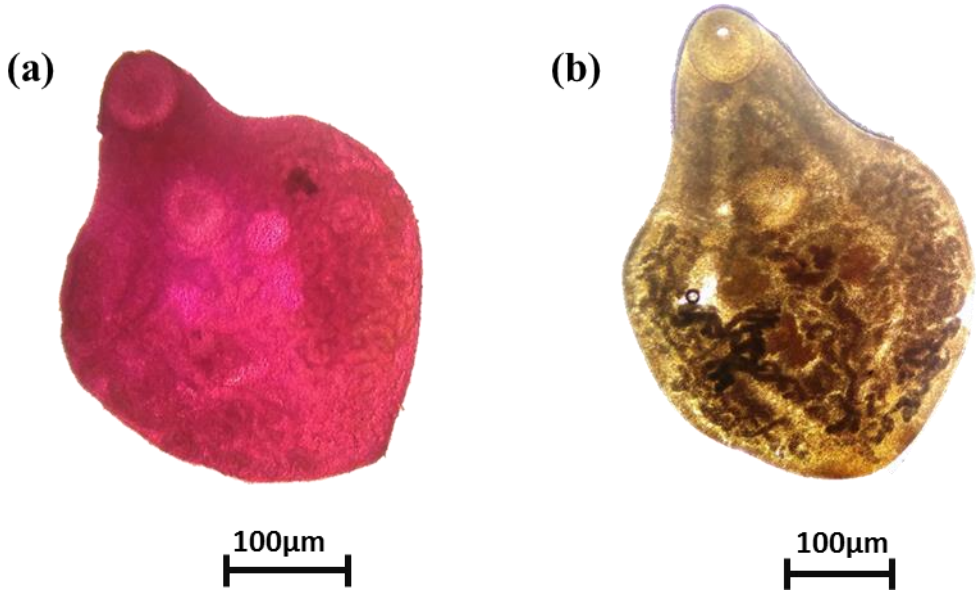

Fig. 1.18. Light microscopic (Leica DM1000) images of *Phyllodistomum* sp. (stained in Borax carmine)

a) & (b) General view of the whole worm

Class: Eoacanthocephala

Order: Gyracanthocephala Van Cleave, 1936

Family: Quadrigyridae Van Cleave, 1920

Genus: *Pallisentis* Van Cleave, 1928

***Pallisentis ophiocephali* (Thapar, 1931) Baylis, 1933**

(Fig. 1.19) (Table 1.17)

Total number of specimens collected: 991

Description: Proboscis short, globular with 4 circles of 10 hooks each surrounding it; lemniscus long, slender and cylindrical; proboscis receptacle cylindrical to saccate, smooth with single layer of the muscular walls reaching to the anterior region of the trunk where the proboscis receptacle is marked off from the trunk with slight demarcation. The posterior extremity is rounded. Testes cylindrical, contiguous; bursa everted, cement glands and ducts long, genital ducts filiform and long. Vulva simple bulge without lips, eggs elliptical.

Host: *Channa striata, Channa punctata* and *Channa gachua*

Habitat of the parasite: Intestine

Habitat of the host: Ponds and lakes

Locality: Imphal East (24.7807° N and 93.9674° E), Imphal West (24.7828° N and 93.8859° E), Thoubal (24.7828° N and 93.8859° E) and Bishnupur (23.0679° N and 87.3165° E).

Sampling Date: 1-15 of every alternate month from August 2014 - July 2017.

Remarks: *Pallisentis ophiocephali* was established by Thapar (1931) in *Ophiocephalus marulius*. Many species of *Pallisentis* including *P. allahabadi*, *P. magnum*, *P. nandai*, and *P. nagpurensis* are conspecific of *P. ophiocephali* therefore they were synonymized as *P.*

ophiocephali by Soota and Bhattacharya (1982). Occurrence of *P. ophiocephali* has also been reported from Channid hosts of Meghalaya, Manipur and Tripura (Jyrwa *et al*., 2016; Mangolsana *et al*., 2016; Koiri and Roy, 2017c; Banerjee *et al*., 2017).

Table 1.17 Morphometric measurements of *Pallisentis ophiocephali* collected from Manipur, India (N= 10)

Characters	Range (in mm)	Mean ± Standard deviation
Body		
Length	12.21-20.10	16.155 ± 5.579
Breadth	0.21-0.41	0.310 ± 0.141
No. Of collar spines	13	13.000 ± 0.00
No. Of trunk spines	20-30	25.000 ± 7.071
Proboscis		
Length	0.50-0.60	0.550 ± 0.070
Width	0.26-0.40	0.330 ± 0.098
Proboscis hooks		
Length	0.06-0.07	0.065 ± 0.007
Width	0.02-0.02	0.020 ± 0.00
Proboscis receptacle	0.50-0.53	0.515 ± 0.021
Lemniscus		
Length	0.40-0.43	0.415 ± 0.021
Breadth	0.07-0.09	0.080 ± 0.014
Cement reservior		
Length	0.29-0.30	0.295 ± 0.007
Breadth	0.04-0.05	0.045 ± 0.007
Bursa		

Length	0.11-0.13	0.120 ± 0.014
Breadth	0.20-0.22	0.210 ± 0.014
Cement glands		
Length	0.32-0.33	0.325 ±0.007
Breadth	0.10-0.11	0.105 ± 0.007
Testes		
Length	1.5-1.7	1.600±0.141
Breadth	0.14-0.16	0.150±0.014

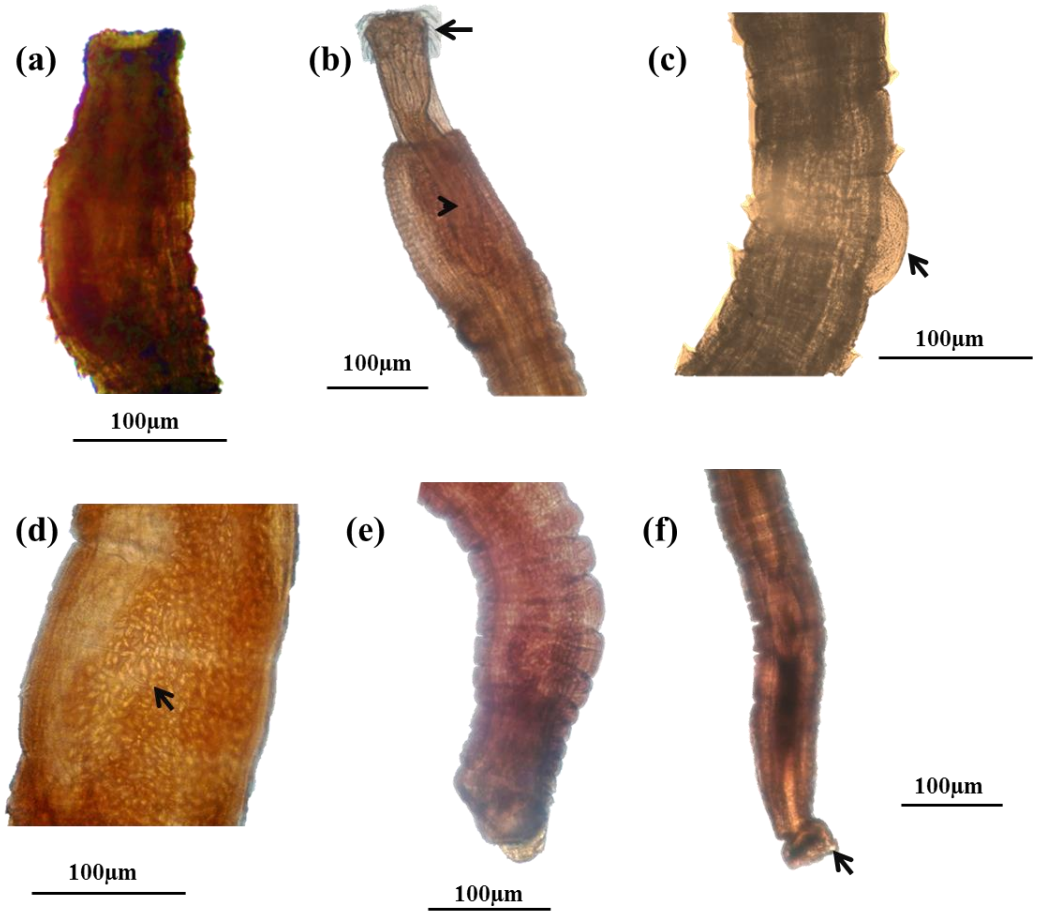

Fig. 1.19 Light microscopic (Leica DM1000) images of *Pallisentis ophiocephali*

a) Proboscis of larval form

b) Anterior region showing proboscis with hooks (arrow) and saccate proboscis receptacle (arrowhead)

c) Posterior third of female body showing vulva (arrow)

d) Enlarged view of uterus filled with eggs (arrow)

e) Tail end of female

f) Tail end of male showing well developed bursa (arrow)

Genus: *Pallisentis* Van Cleave, 1928

***Pallisentis indicus* Mithal and Lal, 1981**

(Fig. 1.20) (Table 1.18)

Total number of specimens collected: 468

Description: Proboscis medium, globular with 4 circles of 10 hooks; sub-equal lemniscus; proboscis receptacle cylindrical to saccate, smooth with single layer of the muscular walls reaching to anterior region of trunk where the proboscis and trunk are continuous and not introverted. Trunk has a collar of spine arranged in 14-16 closely set rings near anterior extremity, posterior to this region is an unspined region, which is followed by 20-40 widely spaced rings of spines and the caudal part is devoid of spines. Testes elongated and vulva located at about middle of the body.

Host: *Channa striata, Channa punctata* and *Channa gachua*

Habitat of the parasite: Intestine

Habitat of the host: Ponds and lakes

Locality: Imphal East (24.7807° N and 93.9674° E), Imphal West (24.7828° N and 93.8859° E), Thoubal (24.7828° N and 93.8859° E) and Bishnupur (23.0679° N and 87.3165° E).

Sampling Date: 1-15 of every alternate month from August 2014 - July 2017.

Remarks: *Pallisentis indicus* was established by Mithal and Lal, 1981. The present form shows maximum similarity with *P. ophiocephali* but differs in few identifying features like number of collar spines and ring of spines in the body, where a number of collar spines are 14-16 in *P. indicus* whereas 13 in *P. ophiocephali* and ring of spines are 20-40 in *P. indicus* whereas 20-30 in *P. ophiocephali*. In the present form proboscis and trunk are in

continuous and not introverted, it has 4 circles of 10 hooks in proboscis and subequal lemniscus. Thus, the present form is identified as *P. indicus.* This species has earlier been reported from Manipur by Mangolsana *et al*. (2016).

Table 1.18 Morphometric measurements of *Pallisentis indicus* collected from Manipur, India (N= 10)

Characters	Range (in mm)	Mean ± Standard deviation
Body		
Length	11.10-18.09	14.59 ± 4.942
Breadth	0.21-0.30	0.255 ± 0.063
No. Of circles of collar spines	15	15.00 ± 0.00
No. Of circle of spines in trunk	20-40	30.00 ±14.142
Proboscis		
Length	0.45-0.55	0.50 ± 0.070
Breadth	0.25-0.35	0.300 ± 0.070
Proboscis hooks		
Length	0.05-0.07	0.055 ± 0.007
Breadth	0.02-0.02	0.020 ± 0.00
Proboscis receptacle	0.50-0.55	0.525 ± 0.035
Lemniscus		
Length	0.38-0.40	0.390 ± 0.014

Breadth	0.07-0.08	0.075 ± 0.007

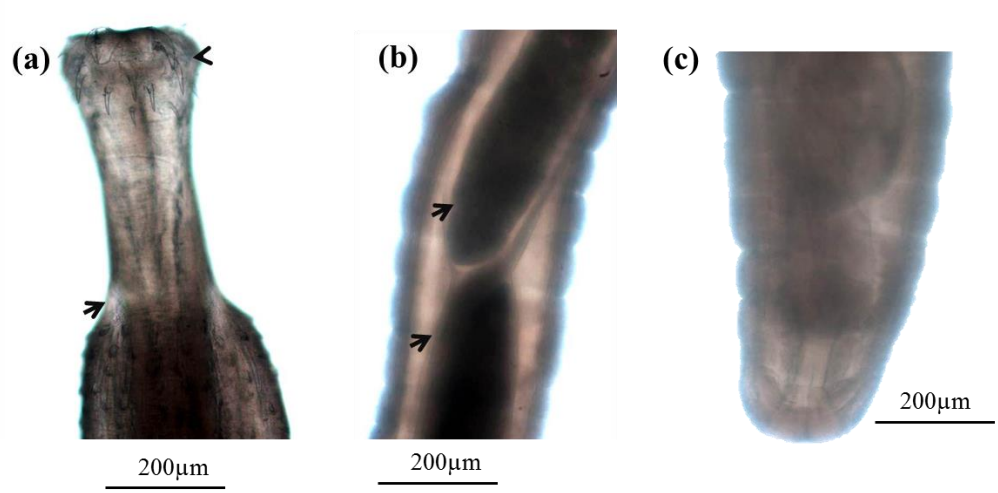

Fig. 1.20 Light microscopic (Leica DM1000) images of *Pallisentis indicus*

a) Anterior region of the body showing proboscis with hooks (arrowhead) and continuous with trunk (arrow)

b) Mid region of the body showing elongated testes (arrows)

c) Posterior end of the body

Genus: *Pallisentis* Van Cleave, 1928

***Pallisentis sp*.**

(Fig. 1.21) (Table 1.19)

Total number of specimens collected: 18

Description: Proboscis medium, globular with 4 circles of 10 hooks; proboscis receptacle stout reaching to anterior region of the trunk where the proboscis is introverted; lemniscus long, slender and cylindrical. Trunk has a collar of spine arranged in 6-10 closely set rings near anterior extremity. Posterior to the collar spines is an unspined region, which is followed widely spaced rings of spines extending to the posterior region of the body; uterine bell visible; testes cylindrical, elongated and contiguous; bursa bulbous.

Host: *Channa striata*

Habitat of the parasite: Intestine

Habitat of the host: Ponds

Locality: Imphal West (24.7828° N and 93.8859° E)

Sampling Date: 9th May, 2015

Remarks: The genus *Pallisentis* was erected by Van Cleave (1928) with *P. umbellatus* as type species from *Ophiocephalus argus, Siniperca spp., Cobitis decemcirrosus* and *Parasilurus asotus* from China. Subsequently, several species of *Pallisentis* have been

described. From India, these include *P. ophiocephali*, *P. nagpurensi*, *P. colisai*, *P. basiri*, *P. pandei*, *P. fasciati*, *P. gomtii*. *P. cavasii*, *P. channai*, *P. vinodai*, *P. punctate* and *P. anandai*, (Yamaguti, 1963; Gupta *et al*., 2015a; Gupta *et al*., 2015b; Gautam *et al*., 2017). The species level identification of this specimen was not possible due to inaccessibility of the previously described species for comparison.

Table 1.19 Morphometric measurements of *Pallisentis* sp. collected from Manipur, India (N= 2)

Characters	Range (in mm)	Mean ± Standard deviation
Body		
Length	9.30-10.25	9.775 ± 0.671
Breadth	0.65-0.69	0.670 ± 0.028
Proboscis		
Length	0.20-0.22	0.210 ± 0.014
Breadth	0.19-0.20	0.195 ± 0.007
Proboscis hooks		
Length	0.05-0.06	0.055 ± 0.007
Breadth	0.02-0.02	0.020 ± 0.000
Proboscis receptacle		
Length	0.26-0.28	0.270 ± 0.014
Breadth	0.40-0.42	0.410 ± 0.014
Lemniscus		
Length	1.20-1.40	1.300 ± 0.141

Breadth	0.35-0.38	0.365 ± 0.021
Eggs		
Length	0.02-0.03	0.025 ± 0.007
Breadth	0.01-0.02	0.015 ± 0.007

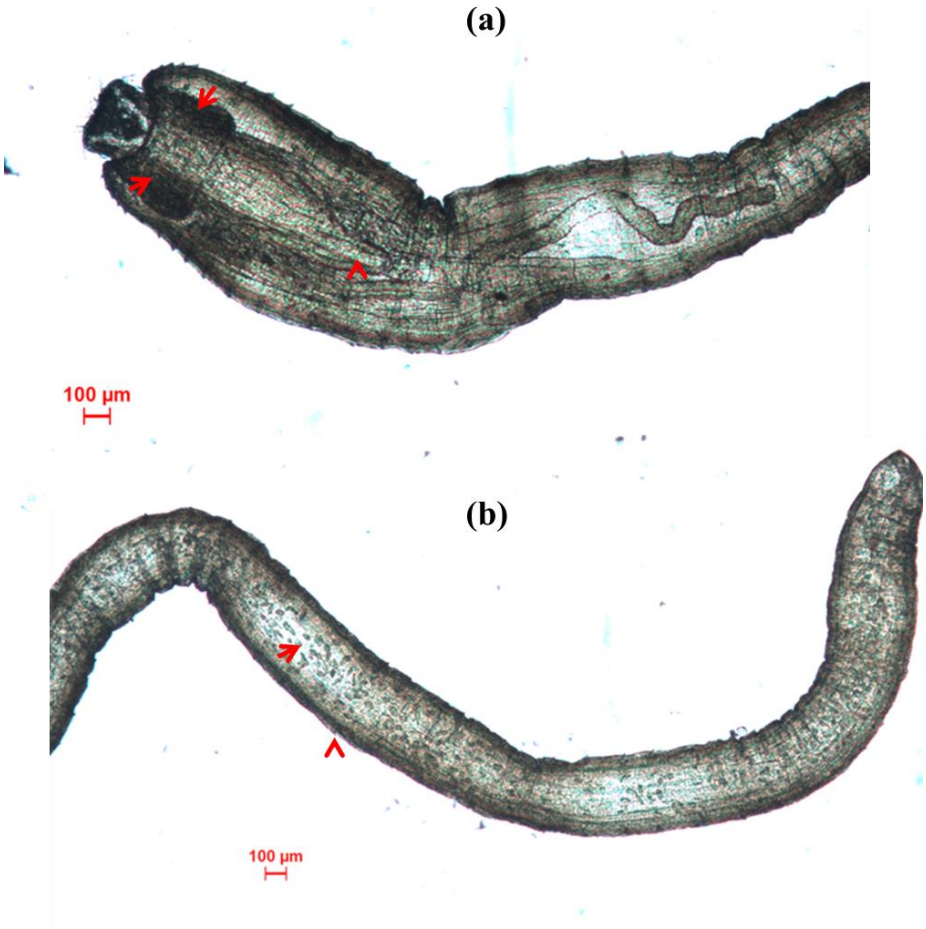

Fig. 1.21 Light microscopic (Leica DM1000) images of *Pallisentis* sp.

a) Anterior region of the body showing stout and bipartite proboscis receptacle (arrows) and long lemniscus (arrowhead)

b) Posterior half of the body showing numerous eggs (arrow) and sparse body spination (arrowhead)

Class- Archiacanthocephala

Order- Echinorhynchida

Family- Echinorhynchidae, Cobbold, 1879

Genus- *Echinorhynchus* Zoega in Muller, 1776

***Echinorhynchus* sp.**

(Fig. 1.22) (Table 1.20)

Total number of specimen collected: 1

Description: Body huge, the anterior region of the body much bigger and gradually decrease towards the posterior end. Proboscis long, cylindrical, with 11 longitudinal rows of 5-16 hooks, each in spiral arrangement; proboscis receptacle claviform, lemniscus saccate. Lacunar system consisting of lateral main vessels and reticular anastomes.

Host: *Channa striata*

Habitat of the parasite: Intestine

Habitat of the host: Pond

Locality: Bishnupur (23.0679° N and 87.3165° E).

Sampling Date: 10th October, 2016.

Remarks: *Echinorhynchus* was established by Muller, 1776 with the type species as *E. gadi*. Yamaguti, 1963 listed 31 species under this genus. The first report of occurrence of *Echinorhynchus* from India is *E. oreintale* by Kaw (1951) in *Schizothorax sp*. from Kashmir. Several species of *Echinorhynchus* have been described from neighbouring countries like china and Japan: It includes, *E. alpinum*, *E. kushiroense*, *E. parasiluri*, *E. cotti* and *E. lotellae* (Yamaguti, 1963). Numerous works on occurrence of *Echinorhynchus* and description of new species have been reported by many authors eg. *E. salmonis* (Muzzal and Mychek-Londer, 2014), *Echinorhynchus sp*. (Sakthivel *et al*., 2014), *E. veli* (Sheema *et al*., 2015), *E. baeri* (Amin *et al*., 2016) and *Echinorhynchus* sp. (Farooq *et al*., 2016). The present study is the first report of the occurrence of the genus *Echinorhynchus* from Manipur, Northeast, India.

Table 1.20 Morphometric measurements of *Echinorhynchus* sp. collected from Manipur, India (N= 1)

Characters	(in mm)
Body	
Length	9.90
Width	1.60
Proboscis	0.44
Length	0.30
Width	
Proboscis hooks	0.07
Length	0.01
Width	
Proboscis receptacle	0.60

Length	0.35
Width	
Lemniscus	2.00
Length	0.40
Width	

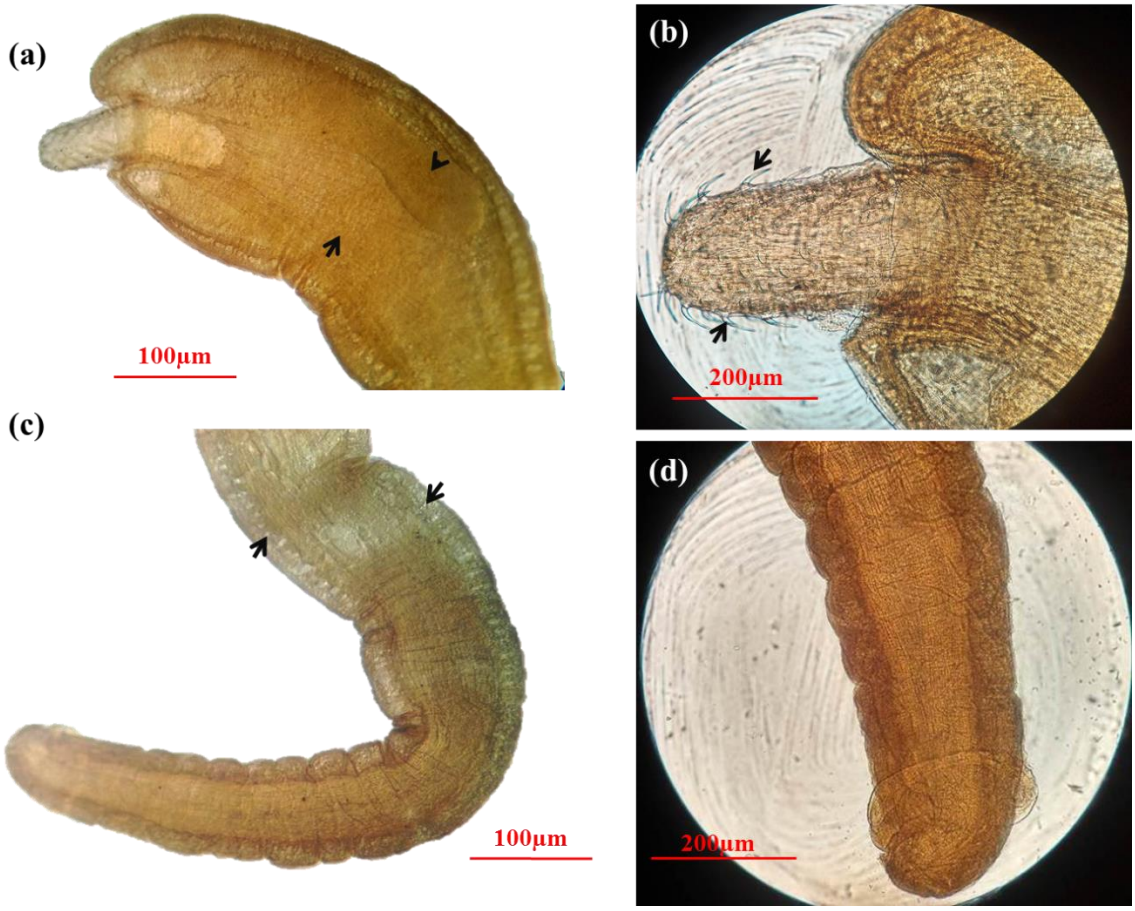

Fig. 1.22 Light microscopic (Leica DM1000) images of *Echinorhynchus* sp.

a) Anterior region of the body showing presence proboscis, claviform receptacle (arrow) and saccate lemniscus (arrowhead)

b) Magnified view of proboscis with the spiral arrangement of hooks (arrows)

c) Posterior regions with lacunar systems (arrows)

d) Enlarged view of the tail end

1.4 Discussion

Table 1.21 List of parasite recovered for the present study

Parasite	**Hosts**
1. *Camallanus anabantis*	*Anabas testudineus*
2. *Neocamallaus singhi*	*Anabas testudineus*
3. *Procamallanus* sp.	*Anabas testudineus*
4**.** *Paracamallanus ophiocephali*	*Anabas testudineus*
5. *Paraquimperia manipurensis*	*Anabas testudineus*
6. *Anisakis* sp.	*Channa gachua*
7. *Lytocestus attenuatus*	*Clarias magur*
8. *Lytocestus indicus*	*Clarias magur*
9. *Lytocestus filiformes*	*Clarias magur*
10. *Lytocestus longicollis*	*Clarias magur*
11. *Djombangia penetrans*	*Clarias magur*
12. *Senga lucknowensis*	*Channa punctata*
13. *Astiotrema reniferum*	*Clarias magur*
14. *Clinostomum philippinense*	*Trichogaster fasciata*
15. *Posthodiplostomum* sp.	*Channa punctata, C. gachua*
16. *Phyllodistomum* sp.	*Channa gachua*
17. *Pallisentis ophiocephali*	*Channa striata, C. punctata, C.gachua*
18. *Pallisentis indicus*	*Channa striata, C. punctata, C.gachua*
19. *Pallisentis* sp.	*Channa striata*
20. *Echinorhynchus* sp.	*Channa striata*

In our present study, identification and characterization of parasite recovered from different edible fishes of Manipur, India is provided. Among the 20 species of helminth parasites recovered in the present study, the genus *Phyllodistomum* and *Echinorhynchus*

are reported for the first time from Manipur, Northeast India. Also, the digenetic trematodes with zoonotic potential, i.e., *Clinostomum philippinense* from *Trichogaster fasciata* are reported herein, but it is different from the previously reported *Clinostomum* species from India i.e., *Clinostomum complanatum,* making the parasite species of our study to be the first report from India. Although there has been no report of human *Clinostomum* infection in India, the occurrence of *Clinostomum* infection in a human in other Asian countries is very common (Hara *et al*. 2014). Zoonotic fish parasites, fish-borne trematode (FBT) infections, in particular, had an adverse effect on human health, where, as many as 40 million people had been infected and they are more prevalent in Asian countries (WHO, 2002). In Asia, this is associated with the culture of eating raw fish or inadequately cooked fish. There are 19 such cases that have been reported only from Japan (Hara *et al*., 2014). Pharyngitis and lacramalitis due to *Clinostomum* infection have been reported from Thailand and Korea too (Tiewchaloern *et al*. 1999; Park *et al*. 2009). In addition to human, freshwater fishes, which is one of the intermediate hosts incur severe damage following heavy infections with metacercariae of *Clinostomum* species, popularly known as "yellow grub" (Shareef and Abidi, 2012).

Other metacercarial infections causing diseases like black spots and white grub by different species of *Posthodiplostomum* are also recovered from Channid fish in our present study. The same host also harboured the adult and metacercarial form of *Phyllodistomum* sp. as co-infection with *Posthodiplostomum*. The genus *Phyllodistomum* is one of the most speciose groups among digeneans commonly found in the urinary bladder of amphibians and fishes (Lewis, 1935; Helt, 2003).

The most common helminth parasites found infecting *Anabas testudineu*s in our collection are the nematodes. Within nematoda, the genus *Camallanus* representing

approximately 27 species are found in freshwater and marine fishes, amphibians and reptiles (Yamaguti, 1961). In a global perspective, the tropical Asian region harbours the greatest diversity of Camallanid fauna (Stromberg and Crites, 1975). Camallanids like (*Camallanus anabantis, Paracamallanus ophiocephali, Procamallanus sp.* and *Neocamallanus singhi*) and Quimperidae (*Paraquimperia manipurensis*) are common in *Anabas testudineus* and other small fishes in India, including Manipur and neighbouring countries (Shomorendra and Jha, 2003; Puinyabati, 2010; Luangphai *et al*., 2012; Jyrwa *et al*., 2016).

The adult and larval form of the genus *Pallisentis* were found infecting channid fishes. *Pallisentis spp*. infestation have been reported from the same fish (Gupta *et al*., 2015; Gautum *et al*., 2017; Banerjee *et al*., 2017;). In the present study, four species of *Pallisentis* are found co-infecting the same host. *Pallisentis* sp, *P. ophiocephali*, and *P. indicus* are similar in having a proboscis with 4 circles of 10 hooks. The differences among these species include protrusible praesoma in case of *Pallisentis* sp. whereas in *P. ophiocephali*, and *P. indicus*, it is non-protrusible; the feature like everted bursa is found to occur only in *P. ophiocephali*. The species of the present forms of *Pallisentis* showed maximum resemblance with the previously identified *Pallisentis* (Yamaguti, 1963 and Mithal and Lal, 1981)**.** Apart from the genus *Pallisentis, Echinorhynchus* is also found to co-infect the channid fishes from different localities but of rare occurrence. Reports of occurrence of *Echinorhynchus* in different fishes were also made by various authors (Muzzal and Mychek-Londer, 2014; Sakthivel *et al*., 2014; Amin *et al*., 2016). This is the first report of the occurrence of the genus *Echinorhynchus* from Manipur.

From the present study, it is known that among the fish-borne helminth parasites, *Clinostomum* metacercariae and *Anisakis* sp. emerged as the potential zoonotic trematode

and nematode respectively, prevailing in Manipur, Northeast India. This study reports the occurrence of twenty species of helminth parasites, out of which seven species (*Neocamallanus singhi*, *Paracamallus ophiocephali*, *Anisakis* sp., *Senga lucknowensis*, *Clinostomum philippinense*, *Phyllodistomum* sp. and *Echinorhynchus* sp.) are reported for the first time from Manipur.

CHAPTER 2
SCANNING ELECTRON MICROSCOPY OF HELMINTH PARASITES

2.1 Introduction

The use of microscopy as a powerful investigative tool in the study of helminth is increasing and has contributed to the better understanding of complex life cycles and host-parasite relationships. Its use in parasitology is not only confined in taxonomy but in the multidisciplinary study of parasites like functional study, epidemiology and disease control (Halton, 2004). In relation to this, the use of scanning electron microscopy in understanding the tegumental nature of parasites has proven to be important in drug efficacy test and drug resistance test (Roy *et al*., 2012; Giri and Roy, 2014).

Identification of helminth parasites is usually done through conventional methods using a light microscope, but with the advancement in technology, SEM has also been used as a complementary tool for morphological identification (Roy and Tandon, 1993). Recently, molecular tools integrated with different microscopical methods have been used and generally accepted for establishing a genuine taxonomic status of the parasites (Ghatani *et al*., 2014; Robles *et al*., 2014). Thus, for structural characterization of helminth parasites, SEM analysis remains one of the most relevant tools in helminthology.

Among helminths, nematodes exhibit a low morphological diversity at optical level, but SEM studies have proved that they have a diverse morphological variation and it effectively removes observational constraints. The SEM helps in comprehensive understanding of surface architecture of helminths, that is head and tail regions, like buccal

cavity, teeth, lips, cuticular ridges or ornamentations, spines papillae, alae, vulva, bursa, spicules in case of nematodes; scolex, bothria, hooks arrangement, the nature of the segmentation in cestodes and oral suckers, acetabulum, holdfast (metacercariae), haptors, sensillary organs etc. in trematodes (Tandon and Roy, 2002; Halton, 2004).

SEM as a complementary tool in helminth taxonomy, including fish parasites, have been exhibited in the work of many authors (In Camallanidae: Santos and Moravec, 2009; Chaudhary *et al*., 2017. In Anisakidae: Quiazon *et al*., 2008; Kanerek and Bohdanowicz, 2009; In Clinostomatae: Gustinelli *et al*., 2010. In Echinorhynchidae: Amin *et al*., 2016).

In the present study, the helminth parasites recovered from edible fishes of Manipur, India was characterized using SEM in order to complement the light microscopic study and ascertain its specific identity. For parasites of the same genus, the tegumental comparisons were made to show their distinctness.

2.2 Materials and methods

Parasite collection

The collection of fish and recovery of parasites were done as mentioned in chapter 1. The collected parasites were washed thoroughly in PBS and fixed in 10% cold neutral buffered formalin (NBF) at 4^0C for 12-18 hrs.

Scanning electron microscopy (SEM)

The fixed specimens were washed thoroughly in phosphate buffered saline (PBS), cleaned meticulously in water to remove the debris attached to the body and dehydrated in ascending grades (30%, 50%, 70%, 90%, 100%) of acetone. The specimens were then immersed in tetramethylsilane [TMS- $(CCH_3)_4$ Si, boiling point 26.3^{o}C, surface tension 10.3 dynes/cm at 20^{o}C] for 10 min and then brought to room temperature to dry (Dey *et*

al., 1989 modified by Roy and Tandon, 1991). The samples were then mounted on brass or aluminium stubs and metal-coated with gold in a fine coat Ion Sputter JFC-1100 (JEOL). Finally, the samples were observed under scanning electron microscope (SEM) JSM 6360 (JEOL) at electron-accelerating voltages ranging between 10-20 kV.

2.3 Observations and Results

Camallanus anabantis

Microtopographical observations showed that the sclerotized plates present in the anterior portion of the sub-lateral region are attached by a small thread-like structure. Anterior end shows the presence of Knob-like processes arranged dorsoventrally. Cuticle striated transversely without ornamentation. Just below the buccal cavity a pair of deirids present on the lateral sides of the body. Small sub-lateral cephalic papillae present on each side of the plate and fairly rounded. Prominent vulva is present at about the mid-region of the body. Digitform mucrons are prominent (Fig. 2.1)

Neocamallanus singhi

Buccal capsule consists of two lateral valves; ventral view of the head is flat and squarish. Sclerotized plate smooth. A relatively big rounded sub-lateral cephalic papillae with tuberculated structure present on each side of the plate. Deirids present below and further away from buccal cavity on each sides of lateral region. Transversely striated cuticle has distinct lateral alae. The mucron has a horn like projections and smooth surface nature. Male: Caudal alae well developed with several pairs of pedunculatated and sessile papillae showing unique arrangement of the papillae where there are 5 pairs of pre-cloacal papillae, 2 sessile cloacal papillae and 5 pairs of post-cloacal papillae. Curved caudal end have ridges. Dorsal surface of tail end has specifically designed ridges. Enlarged view of the spicule indicates smooth nature of surface (Fig. 2.2).

Paracamallanus ophiocephali

Buccal capsule consisting of large chitinous teeth; chitinous teeth are conical in shape and arranged in rows. Cuticle highly annulated, annulation increases as it approaches the posterior end of the body. Pair of deirids present below the buccal capsule on the lateral sides of the body; tridents prominent and smooth. Tail conical and mucron undivided (Fig. 2.3)

Paraquimperia manipurensis

Body small with longitudinal ridges, slender, anterior end curved dorsally; head region shows presence of three prominent lips where each lip has shallow depression or mild folding which gives an appearance of six lipped structures. Cervical alae prominent which is flap-like structure and relatively big. Female: vulva smooth with two prominent lips, tail unornamented and simple and blunt. Male: Tail tapered to a fine point; 10 pairs of caudal papillae present with a unique arrangement i.e., 4 pairs of pre-anal, 1 pair adanal and 5 pairs of post-anal (3 pairs are sub-ventral, 2 pairs are located laterally). (Fig. 2.4).

***Procamallanus* sp.**

Medium sized nematodes with smooth cuticle. Body elongated and slender; identifying characters of the member of the family Camallanidae, like, tridents at the dorsoventral side of buccal cavity, deirids, cephalic papillae, buccal teeth and amphids are absent; buccal capsule present; sclerotized plate inconspicuous. Body not striated or annulated but smooth. Tail conical, its tip bluntly pointed, curved; caudal alae present (Fig. 2.5).

***Anisakis* sp.**

Body small; head region showed a presence of a pair of boring tooth. Magnified view of boring tooth shows that it has a pointed tip and partially curved. Body tegument smooth, cuticle of tail end is transversely striated. Small excretory pore present at the anterior region and magnified view indicates that the surrounding tegument has rough nature of surface. Cloacal opening prominent at the posterior region of the body and enlarged view indicate the smooth nature of surface; The caudal region transversely striated and tapered into fine end with numerous unoriented papillae where some papillae are small and rounded whereas one papilla showed tuberculation and comparatively bigger than the rest (Fig. 2.6).

Lytocestus attenuatus

Scolex without hooks, simple and not differentiated; neck fairly long; Body slender, elongated, posterior end broader than anterior end. Tegument highly wrinkled in the anterior portion whereas the posterior part of the body shows pseudosegment-like division and the microtriches are filamentous. Excretory pore terminal and smooth (Fig. 2.7).

Lytocestus indicus

Body large and flat, tegument deeply folded transversely and covered with rough papillary structure; scolex rounded to spatulated, unarmed and stout. Neck narrow, undifferentiated and not marked off from the body. Genital pore prominent at the posterior half of the body. Excretory pore terminal and smooth (Fig. 2.8).

Lytocestus filiformes

Body flat, elongated, filiform, posterior end broader than the anterior end. Scolex unarmed, undifferentiated and blunt having specific type of surface folding. Neck elongated and slender. Tegument highly wrinkled and covered with scale-like structure having pointed tips directed posteriorly. Excretory pore terminal (Fig. 2.9).

Lytocestus longicollis

Scolex tumid, spatulate or oblong undifferentiated and unarmed; neck exceedingly long, occupying one third of the body length; body proper short and stout. Body tegument deeply folded with specific pattern of folding and has a smooth surface texture. Genital pore absent. Excretory pore terminal having transverse folds surrounding it (Fig. 2.10).

Djombangia penetrans

Body small, broad and has maximum breadth in the mid-region of the body; scolex small, unarmed and apex is small whereas base of the scolex is broader; neck distinct with longitudinal folds. Body unsegmented, highly wrinkled and folded with smooth surface tegument. Excretory pore terminal (Fig. 2.11).

Senga lucknowensis

Bothria is rectangular with well-developed margins and hollowed at the lateral sides; apical disc present and having specific tegumental folds, centered towards the tip. Body long, metamerism present and non-overlapping; neck absent. Tegument wrinkled having numerous scale-like papillae with pointed tip and directed posteriorly (Fig. 2.12).

Clinostomum Philippinense

Body dorsally convex. The color-like rim of the oral sucker is thick with barely perceptible protrusions; without any papillae and the internal surface of the oral sucker has an uneven appearance. The ventral sucker is surrounded by a rim of tegumental ridges and is divided internally by a thin layered structure; the ventral part of the body has a papillary

tegument and an genital pore is also present. Antero-lateral tegument is spinated while postero-lateral tegument has a cobblestone like pappilae structure. The posterior part of the fluke ends in excretory pore surrounded by relatively long spines (Fig. 2.13).

Posthodiplostomum sp.

The excysted metacercaria has distinct division of body into two parts; forebody elongated. Oral sucker inconspicuous and acetabulum not well-formed. Hind body short cylindrical to oval and surface is smooth. Body tegument in the anterior part is folded (Fig. 2.14).

***Phyllodistomum* sp.**

Body spatulated, hind body broad and cordate shaped; forebody relatively very short, tapering gradually towards the anterior end. Oral sucker slightly bigger than the acetabulum and acetabulum situated slightly in front of the mid-body. Acetabulum prominent having a muscular smooth ring surrounding it. Body tegument showed the presence of raised pitcher-like pores in the papillae. Excretory pore terminal and in the adult forms the eggs were observed surrounding the pore (Fig. 2.15).

Pallisentis ophiocephali

Proboscis not introverted but set off from body proper and globular in shape with 4 circles of 10 hooks each surrounding it. The trunk has a collar spines arranged in 13 closely set rings near anterior extremity. Further back to this collar of spines is a very small gap of unspined region, which is followed by 20-30 widely spaced rings of spines where the spination reduces from mid-region towards the posterior and the caudal part is completely devoid of spines. The bursa protrudes outward at the tail end (Fig. 2.16).

Pallisentis indicus

Proboscis continuous with the body proper and the border between the proboscis and trunk is not well-differentiated. Proboscis is globular with 4 circles of 10 hooks; the collar of spine arranged in 14-16 closely set rings near anterior extremity and the spines are well-oreinted. Vulva is located about middle of the body. Spines present on the mid-body have more pointed tips than the spine on the color of trunk. Spination lessens towards the posterior part of the body and the tail end is devoid of spines (Fig. 2.17).

***Pallisentis sp*.**

Body medium, proboscis very short and stout, somewhat globular, 4 circles of 10 hooks. Body spinose, where the spine lessens towards the posterior region and the posterior end is devoid of spines. The body spines have a pointed tip. Anterior region is broader than the posterior region. Genital pore terminal (Fig. 2.18).

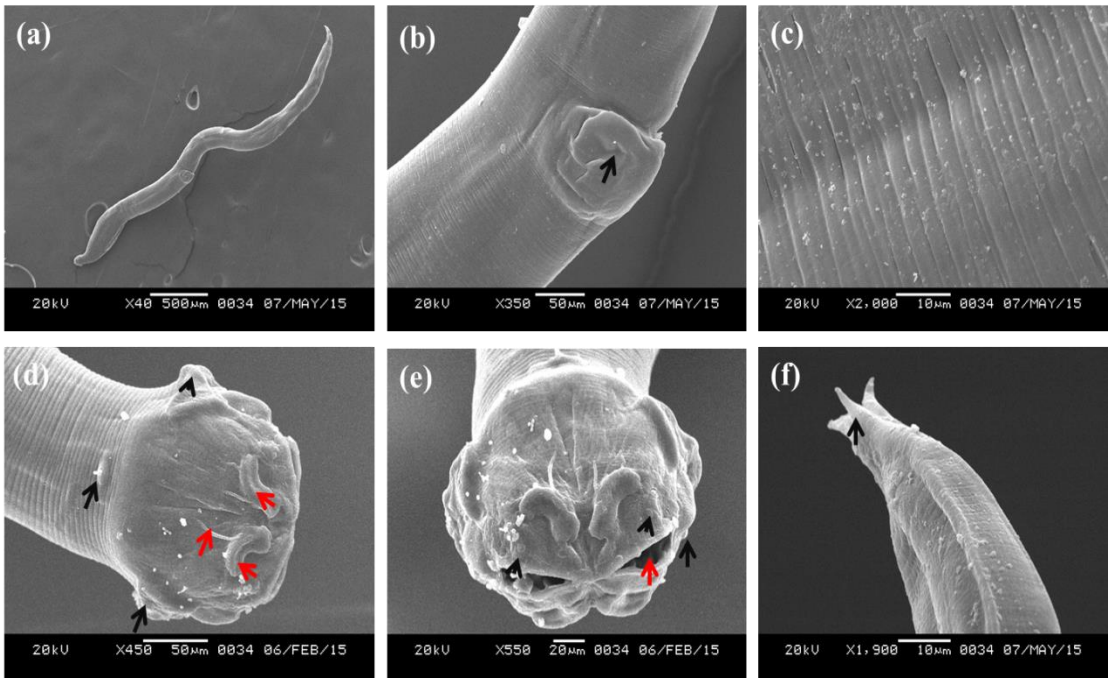

Fig. 2.1 Scanning electron microscopic (JSM 6360, JEOL) photographs of *Camallanus anabantis* showing surface architecture

a) General view of whole nematode.

b) Pre-equatorial portion of female body showing the presence of vulva (arrow).

c) Cuticle showing transverse striations.

d) Anterior end showing presence of deirid (arrow) knob-like processes dorsoventrally (arrowhead) and sclerotized plate with thread-like structure (indicated by arrows in red).

e) Magnified view of buccal capsule with cephalic papillae (arrowheads) longitudinal ridges (arrow in red) and amphid (arrow).

f) Nature of digitiform mucron.

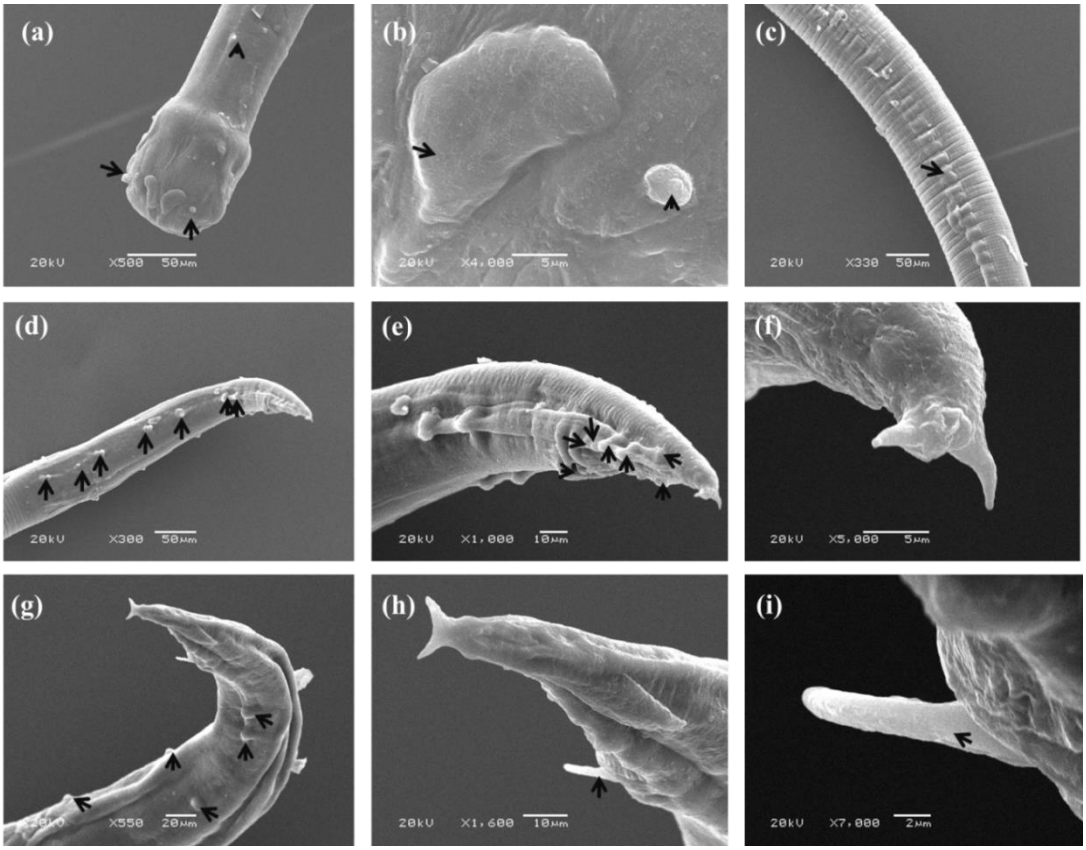

Fig. 2.2 Scanning electron microscopic (JSM 6360, JEOL) photographs of *Neocamallanus singhi* showing surface architecture

a) Dorsal view of anterior end showing deirid (arrowhead) cephalic papillae (arrows).

b) Magnified view of sclerotized plate (arrow) and cephalic papilla (arrowhead)

c) Body showing tuberculated lateral alae (arrow).

d) Caudal end of male showing pre-anal papillae (arrows).

e) Tail region of male showing anal opening (arrowhead) and post-anal papillae (arrows).

f) Mucron showing horn-like structure

g) Lateral view of tail end showing papillae (arrows)

h) Posterior region with spicule (arrow).

i) Magnified view of spicule (arrow)

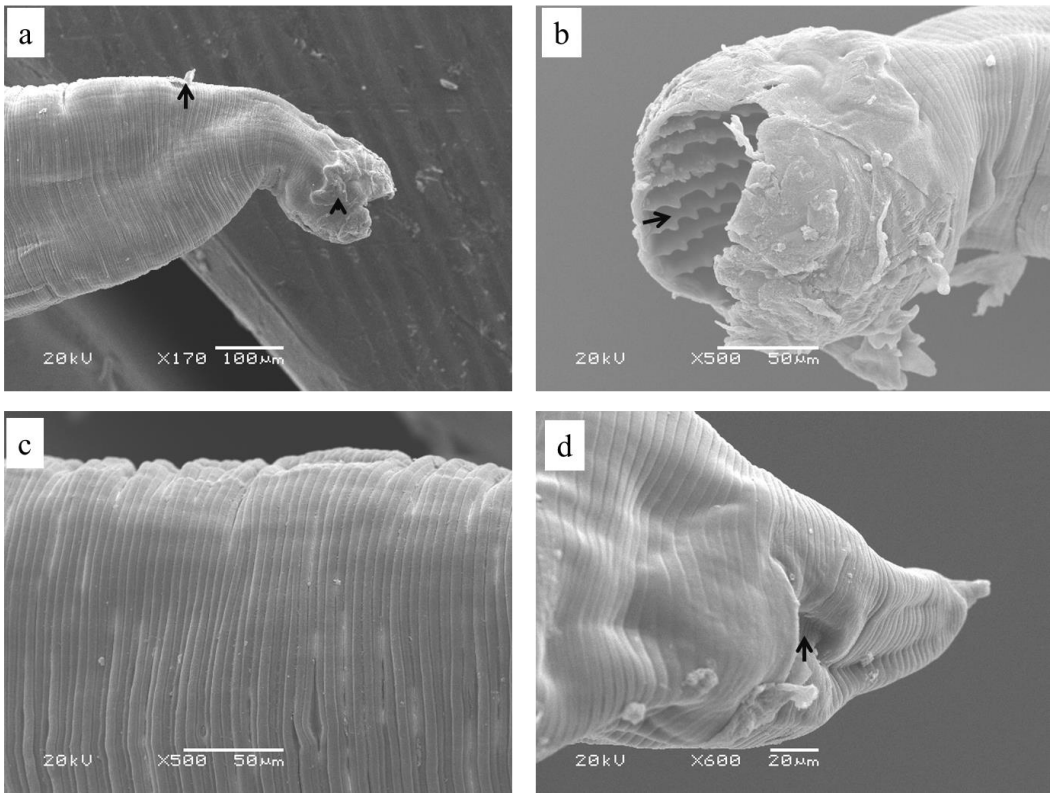

Fig. 2.3 Scanning electron microscopic (JSM 6360, JEOL) photographs of *Paracamallanus ophiocephali* showing surface architecture

a) Anterior end showing trident (arrowhead) and deirid (arrow)

b) Magnified view of buccal cavity showing presence of teeth (arrow)

c) Body showing annulations

d) Posterior end of female showing surface annal opening (arrow) and surface ridges

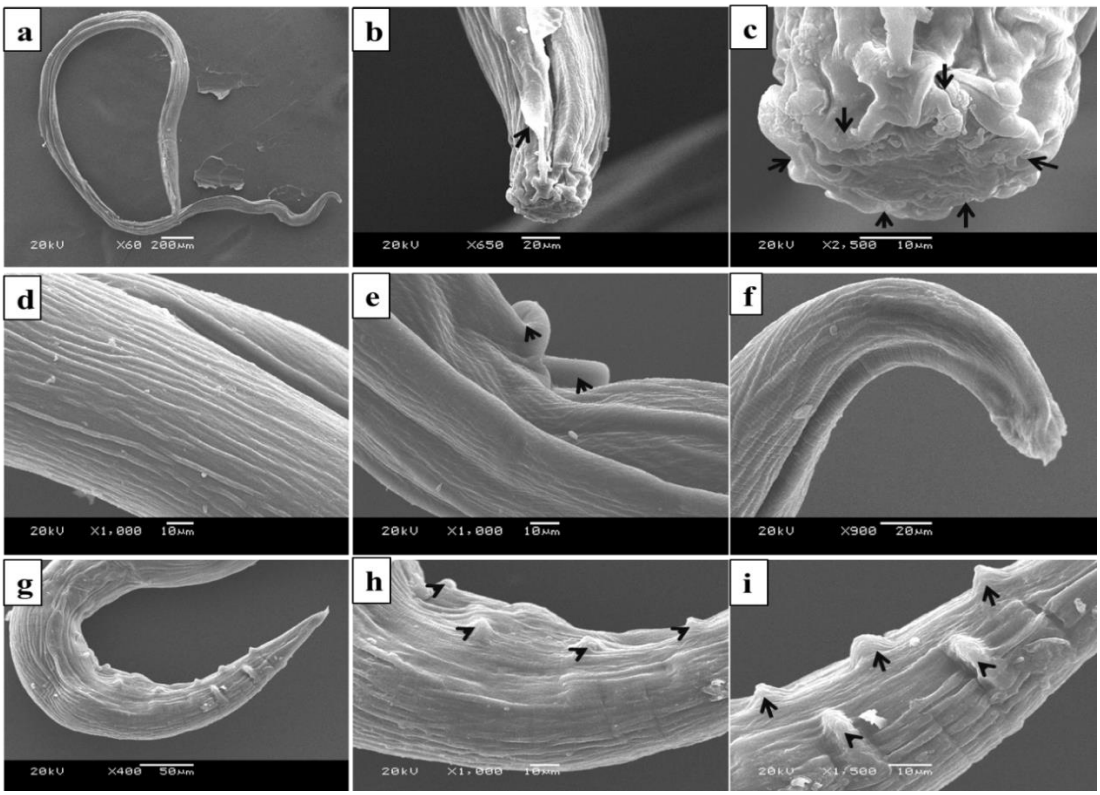

Fig. 2.4 Scanning electron microscopic (JSM 6360, JEOL) photographs of *Paraquimperia manipurensis* showing surface architecture

a) General view of whole nematode.

b) Anterior region with prominent lateral alae having smooth surface (arrow)

c) Magnified view of apical region showing presence of three lips, each lip having a shallow depression/folding giving an appearance of six-lipped structure (arrows)

d) Body cuticle bearing longitudinal ridges

e) Posterior third of the body showing presence of vulva with two prominent lips (arrows)

f) Tail end of adult female.

g) Caudal end of male with numerous papillae

h) Magnified view of pre-anal papillae (arrowhead)

i) Magnified view of post anal papillae [3 sub-ventral (arrow) and 2 lateral (arrowhead)]

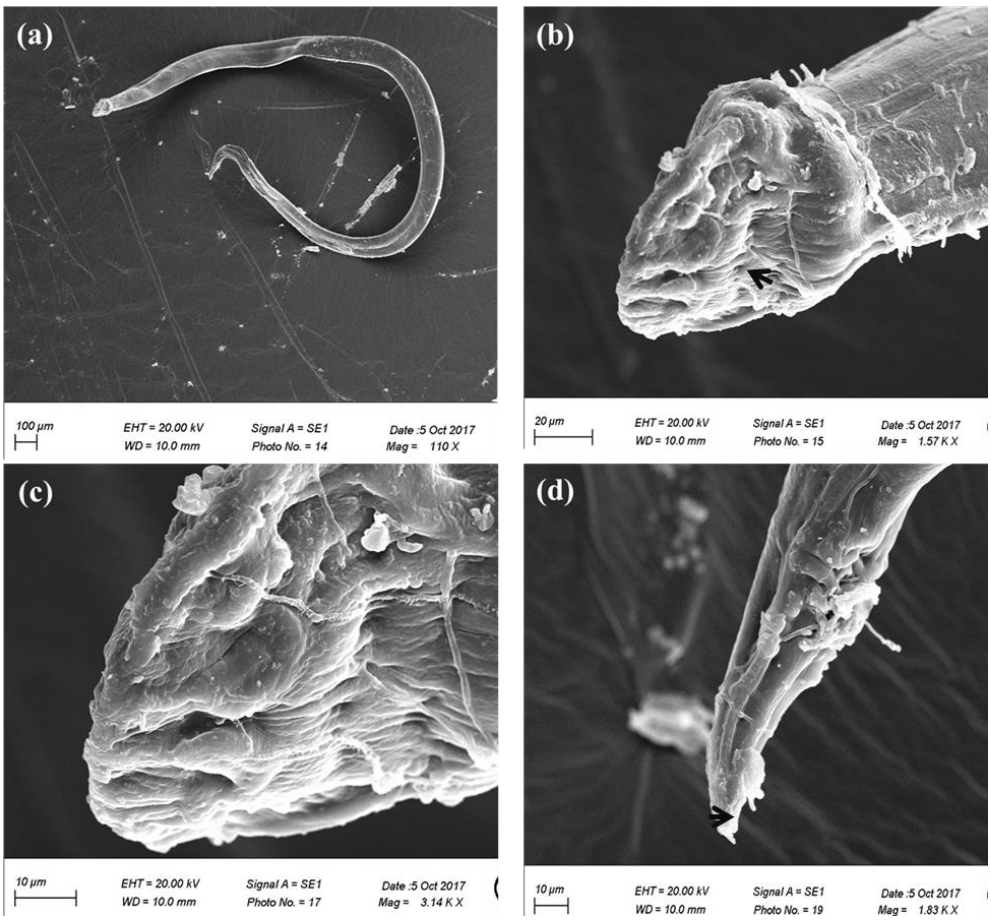

Fig. 2.5 Scanning electron microscopic (JSM 6360, JEOL) photographs of *Procamallanus sp.* showing surface architecture

a) General view of whole nematode

b) Anterior end showing buccal capsule (arrow)

c) Magnified view of dorsal buccal capsule

d) Posterior region showing mucron (arrow)

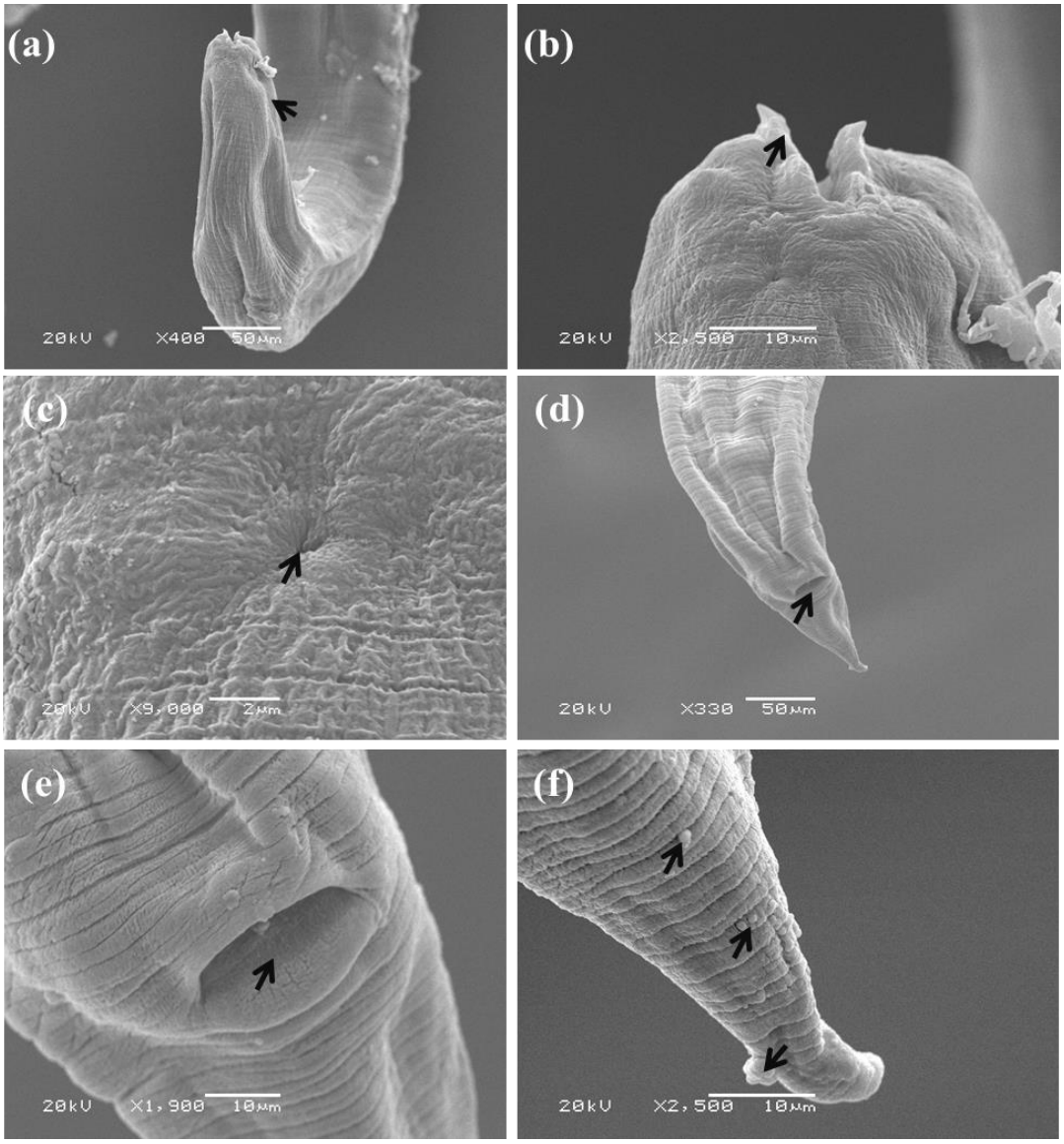

Fig. 2.6 Scanning electron microscopic (JSM 6360, JEOL) photographs of *Anisakis* sp. showing surface architecture

a) Anterior region (arrow)

b) Magnified view of head region showing boring tooth (arrow)

c) Magnified view of the excretory pore present at the anterior region (arrow)

d) Tail end showing cloacal opening (arrow)

e) Magnified view of cloacal opening showing fine topography of inner wall

f) Lateral view of tail showing numerous unoriented papillae (arrows)

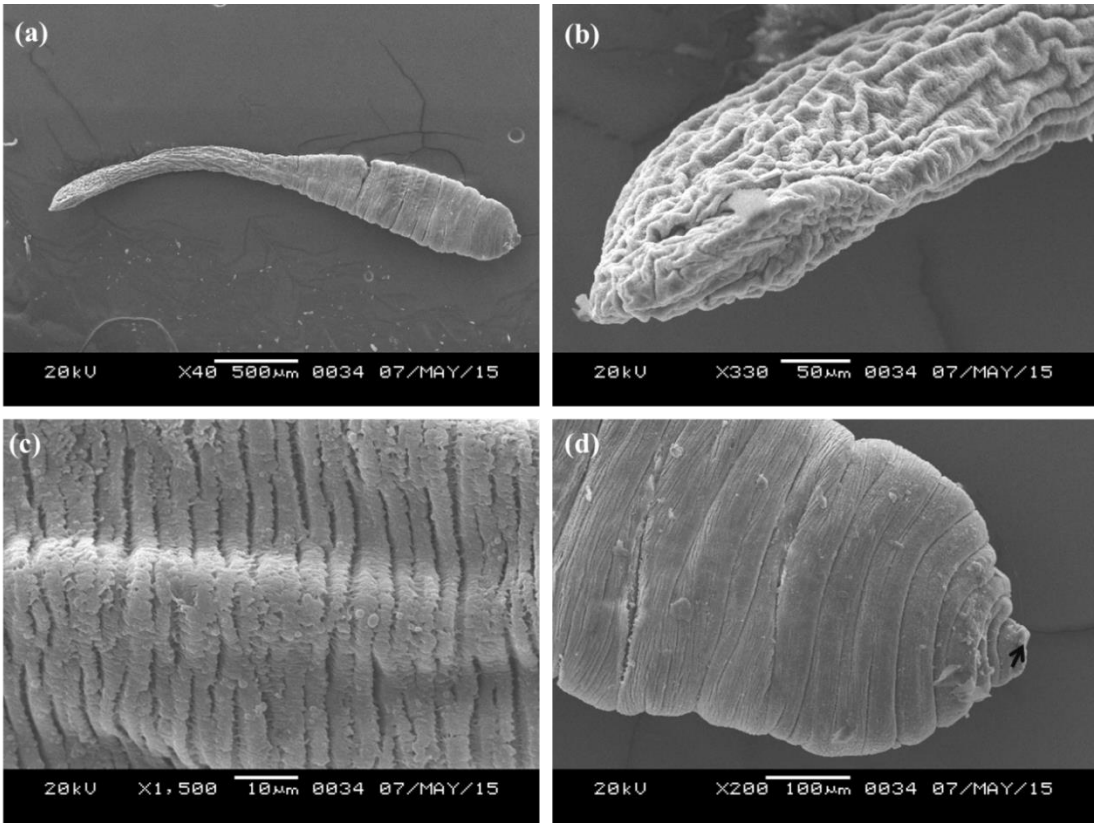

Fig. 2.7 Scanning electron microscopic (JSM 6360, JEOL) photographs of *Lytocestus attenuatus* showing surface architecture

a) General view of the whole body

b) Magnified view of anterior region showing nature of surface topography

c) Body tegument showing transverse folding

d) Posterior region showing terminal excretory pore (arrow)

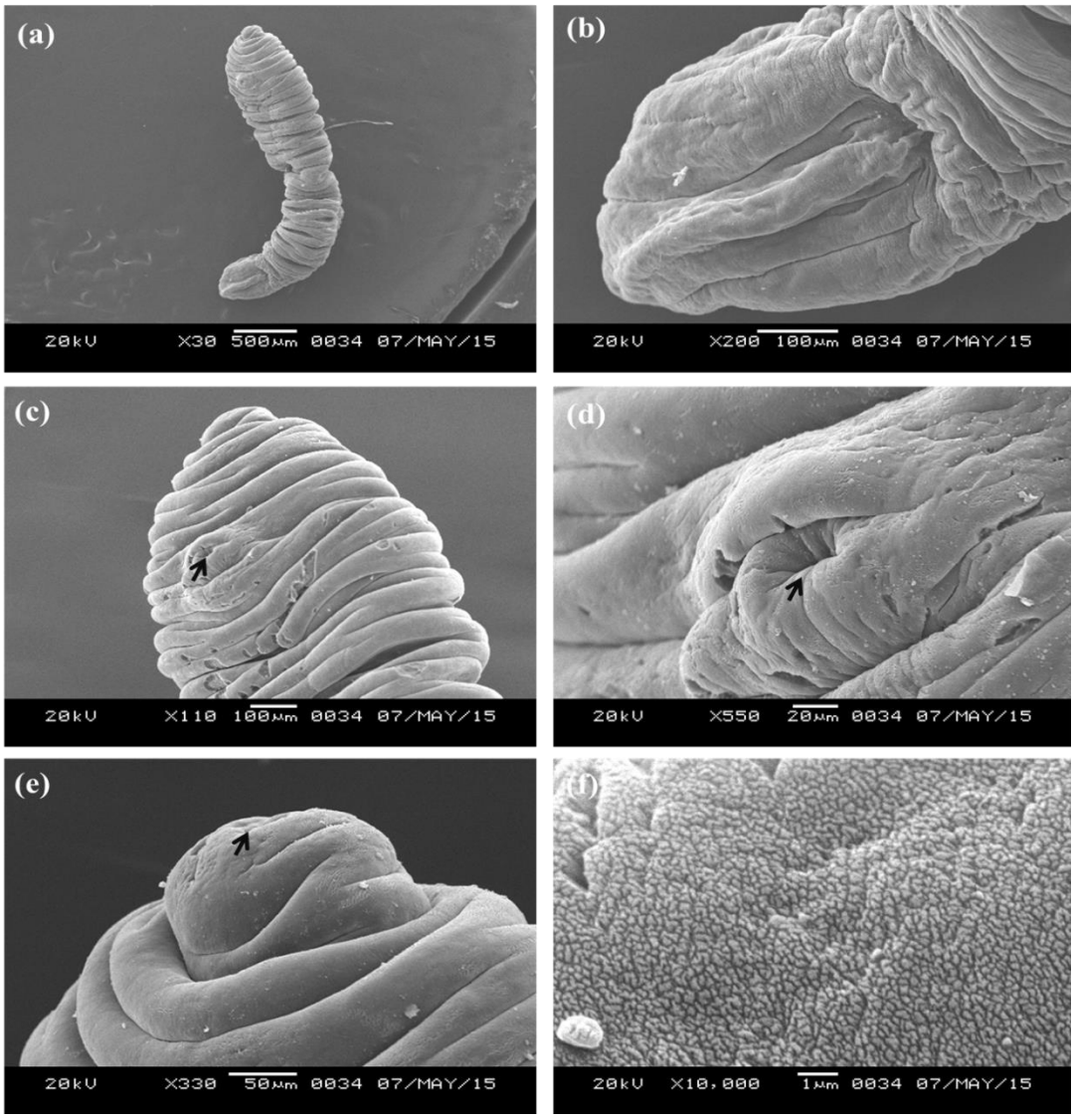

Fig. 2.8 Scanning electron microscopic (JSM 6360, JEOL) photographs of *Lytocestus indicus* showing surface architecture

a) General view of the whole body

b) Magnified view of anterior region

c) Posterior region with traces of segmentation and presence of genital pore (arrow)

d) Magnified view of genital pore (arrow)

e) Posterior end showing terminal excretory pore (arrow)

f) Enlarged view of body tegument showing mosaic arrangement of papillary structure

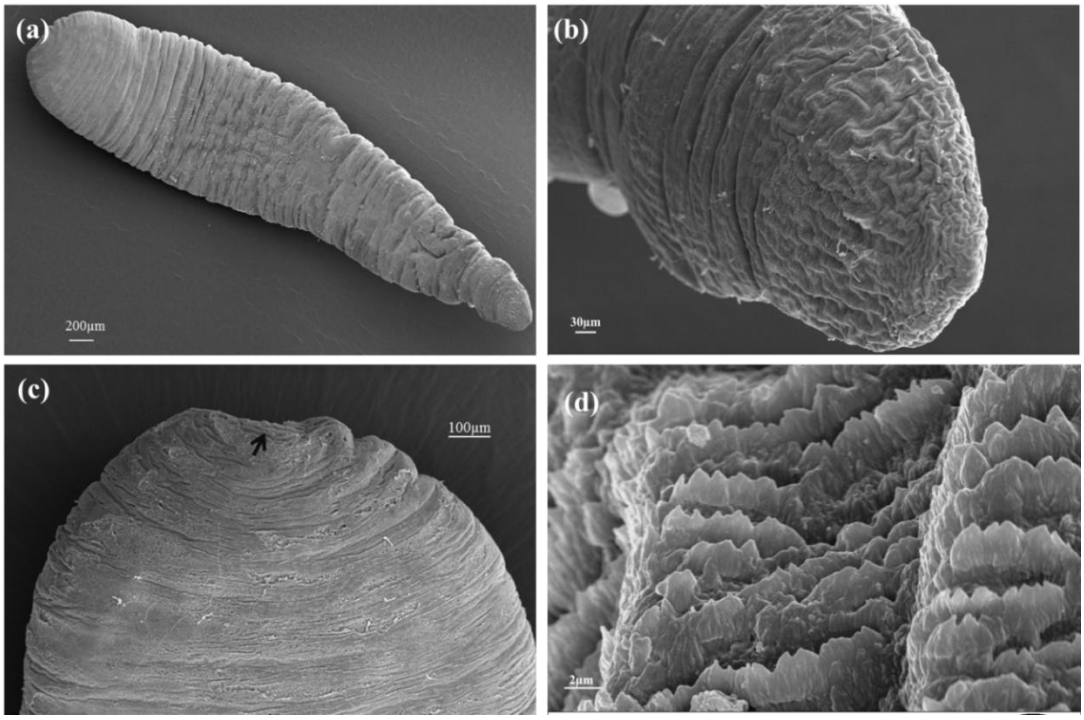

Fig. 2.9 Scanning electron microscopic (JSM 6360, JEOL) photographs of *Lytocestus filiformes* showing surface architecture

a) General view of the whole body

b) Posterior end of the body with terminal excretory pore (arrow)

c) Magnified view of scolex

d) Rough scale-like body tegument having pointed tips arranged in rows

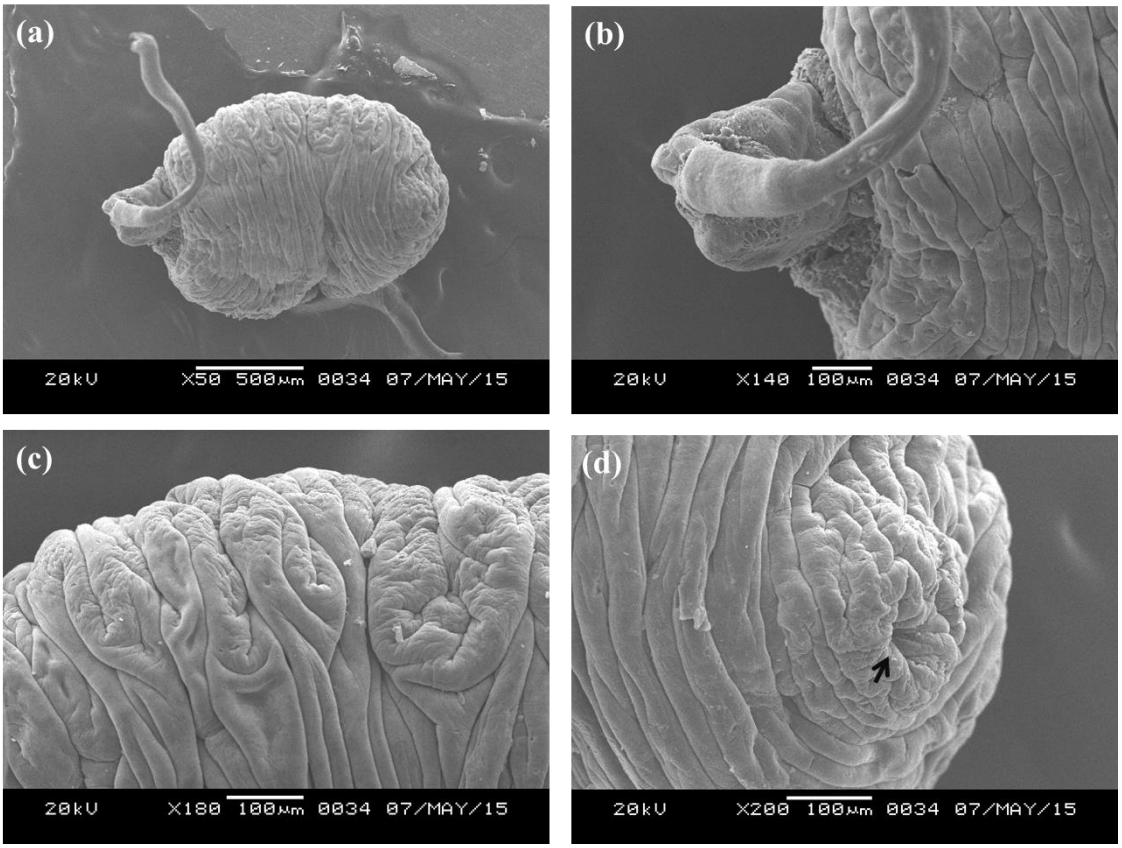

Fig. 2.10 Scanning electron microscopic (JSM 6360, JEOL) photographs of *Lytocestus longicollis* showing surface architecture

a) General view of the whole body

b) Anterior region showing connecting region between the neck and the body proper

c) Magnified view of the body tegument showing specific pattern of tegumental folds

d) Posterior end showing terminal excretory pore (arrow) with surrounding transverse fold having smooth surface

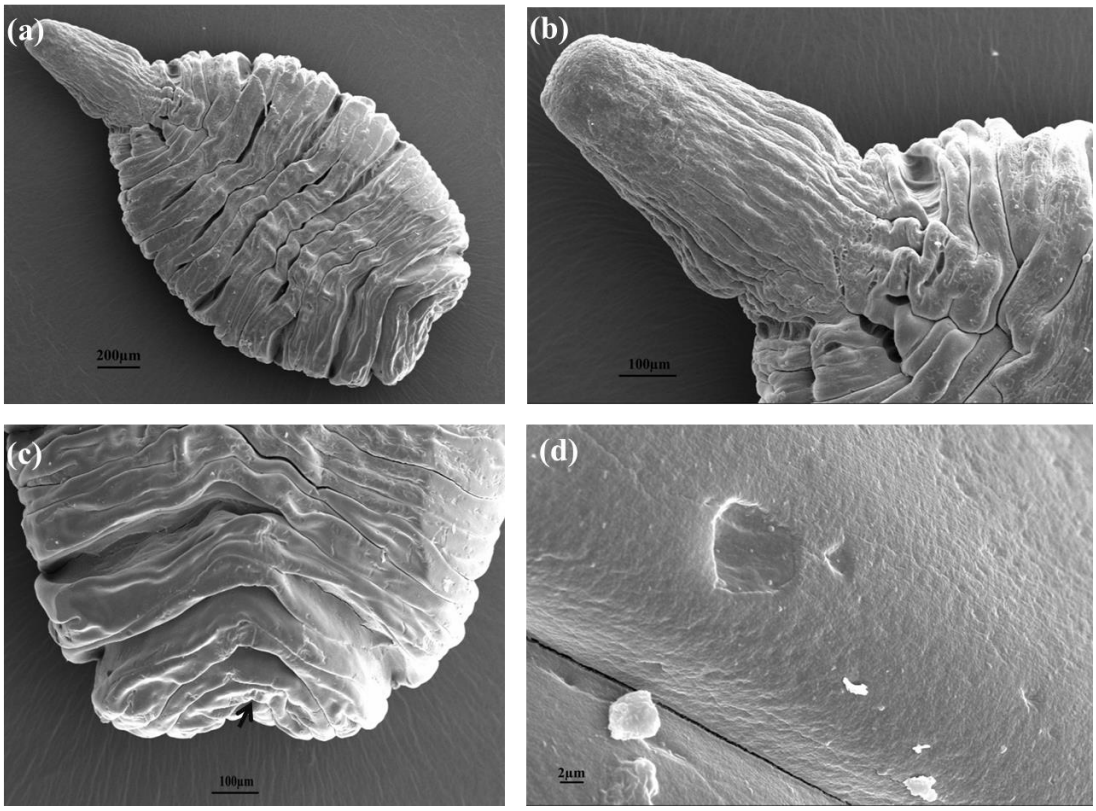

Fig. 2.11 Scanning electron microscopic (JSM 6360, JEOL) photographs of *Djombangia penetrans* showing surface architecture

a) General view of the whole body

b) Anterior region of the body with scolex

c) Posterior end showing terminal excretory pore (arrow)

d) Enlarged view of body tegument showing smooth nature of fine surface

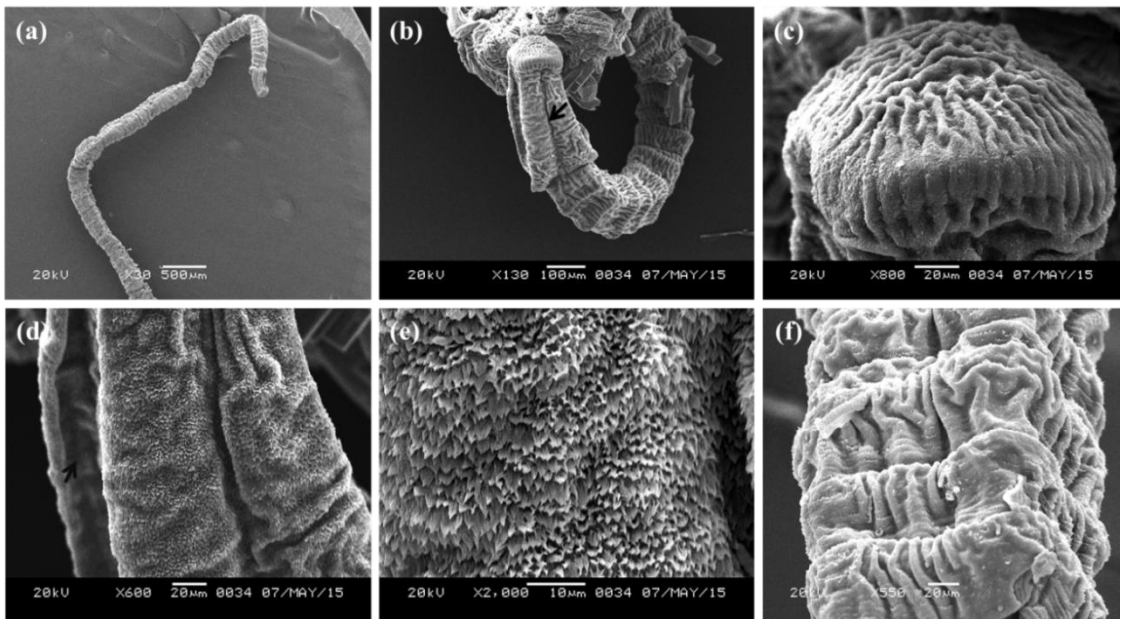

Fig. 2.12 Scanning electron microscopic (JSM 6360, JEOL) photographs of *Senga lucknowensis* showing surface architecture

a) General view of the whole body

b) Anterior region showing rectangular bothria (arrow)

c) Magnified view of apical disc showing specific arrangement of disc surface

d) Bothria with well-developed margins and hollowed lateral sides (arrow)

e) Body tegument showing scales like posteriorly directed papillae

f) Mid-region showing body metamerism and longitudinal tegumental folds

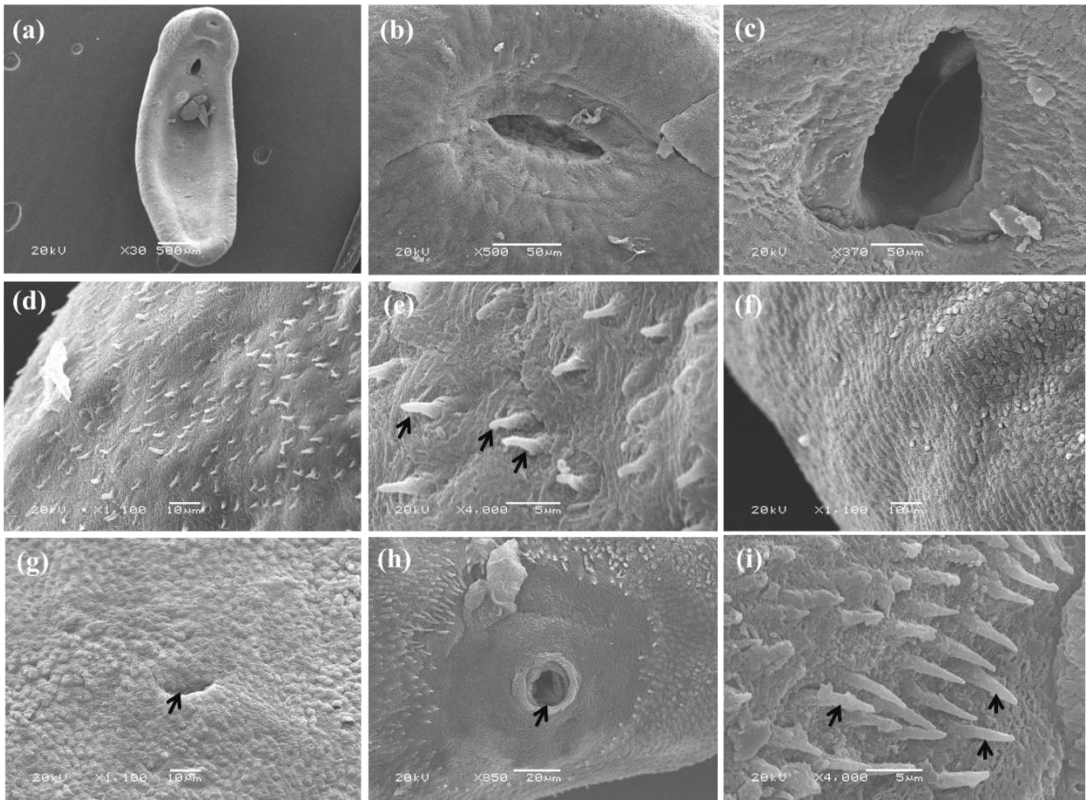

Fig. 2.13 Scanning electron microscopic (JSM 6360, JEOL) photographs of *Clinostomum philippinense* showing surface architecture

a) Ventral view of the whole body

b) Close-up view of oral sucker showing a rim surrounding the sucker

c) Magnified view of acetabulum showing ridges surrounding it

d) Spinated antero-lateral tegument

e) Magnified view antero-lateral spines (arrows)

f) Postero-lateral tegument showing cobblestone like structure

g) Ventral median part of body showing papillary surface topography surrounding the genital pore (arrow)

h) Excretory pore in posterior end of the body (arrow) surrounded by spines

i) Enlarged view of spines surrounding the excretory pore (arrows)

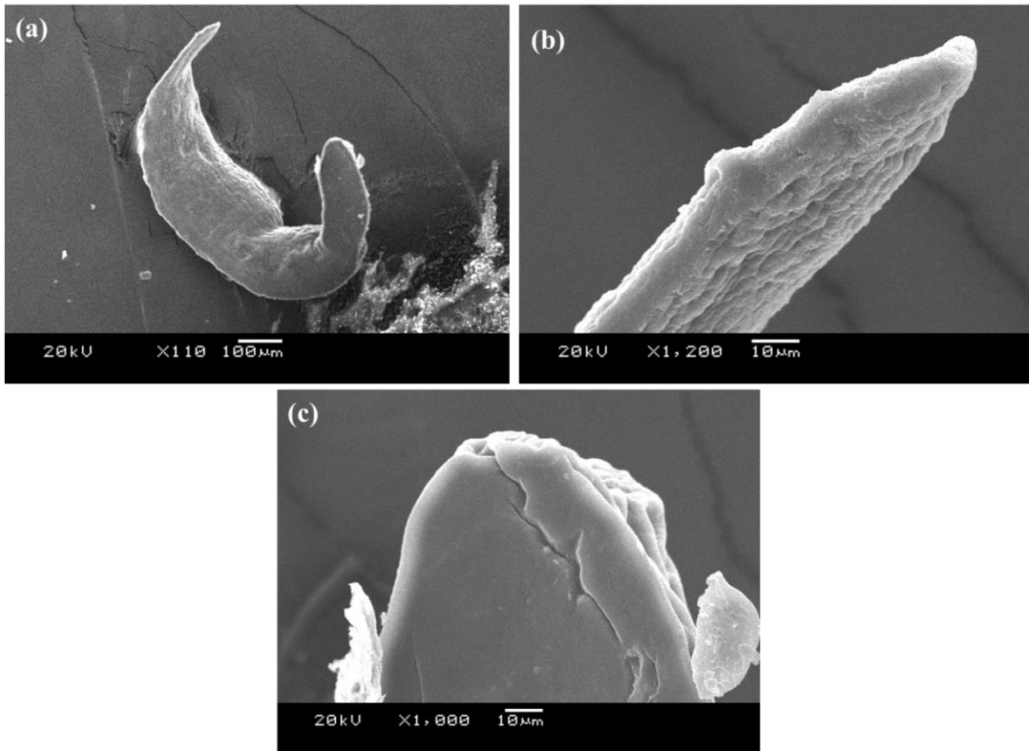

Fig. 2.14 Scanning electron microscopic (JSM 6360, JEOL) photographs of *Posthodiplostomum sp.* showing surface architecture

a) General view of the whole body

b) Anterior end showing nature of tegumental fold

c) Posterior end showing smooth nature of tegument

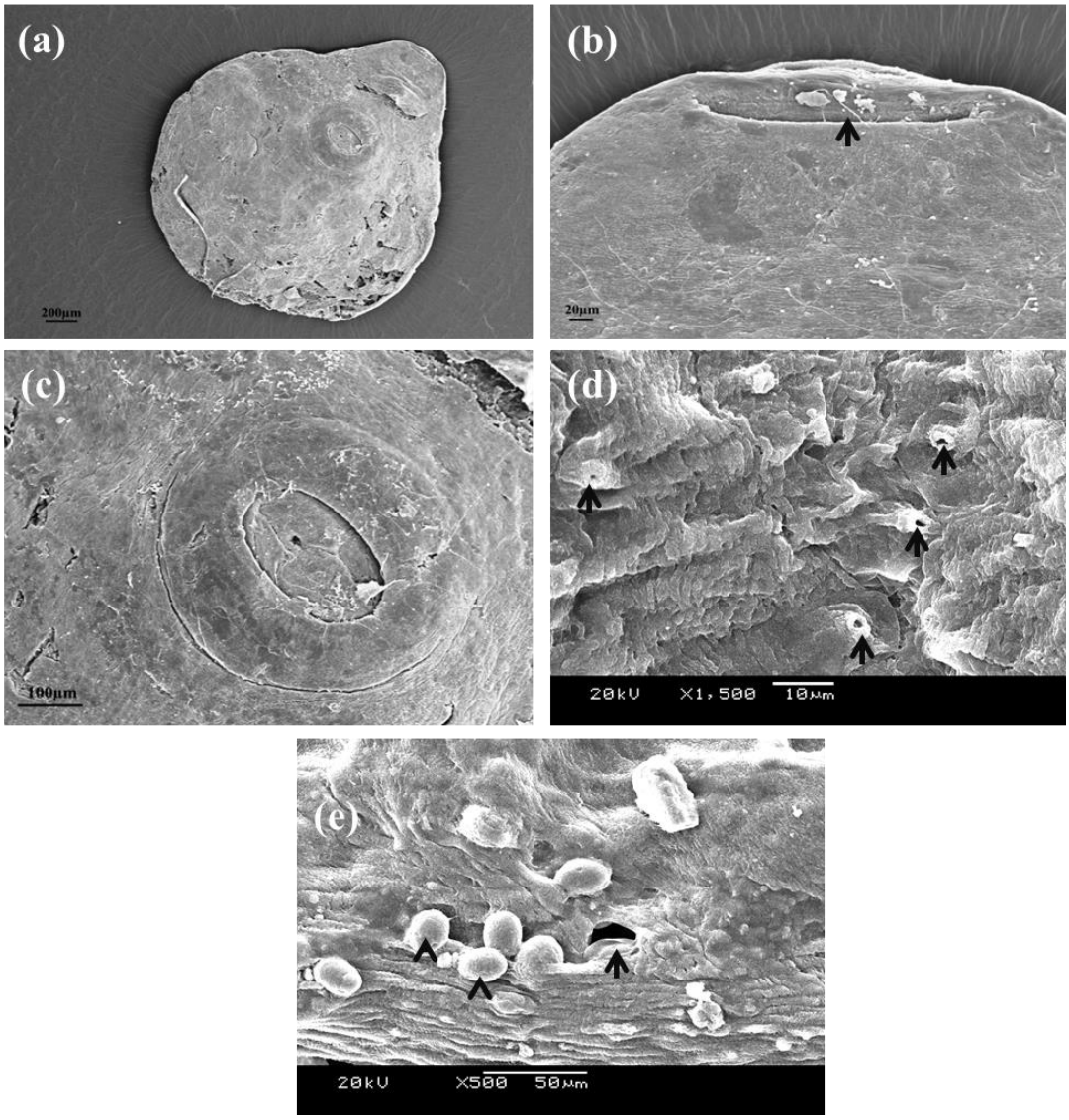

Fig. 2.15 Scanning electron microscopic (JSM 6360, JEOL) photographs of *Phylodistomum sp.* showing surface architecture

a) General view of the adult worm

b) Anterior end with terminal oral sucker (arrow) having smooth tegument surrounding it

c) Enlarged view of acetabulum showing distinct ring surrounding it

d) Body tegument showing presence of pitcher-like pore (arrows) in each raised papillae

e) Posterior end with excretory pore (arrow) and eggs (arrowheads)

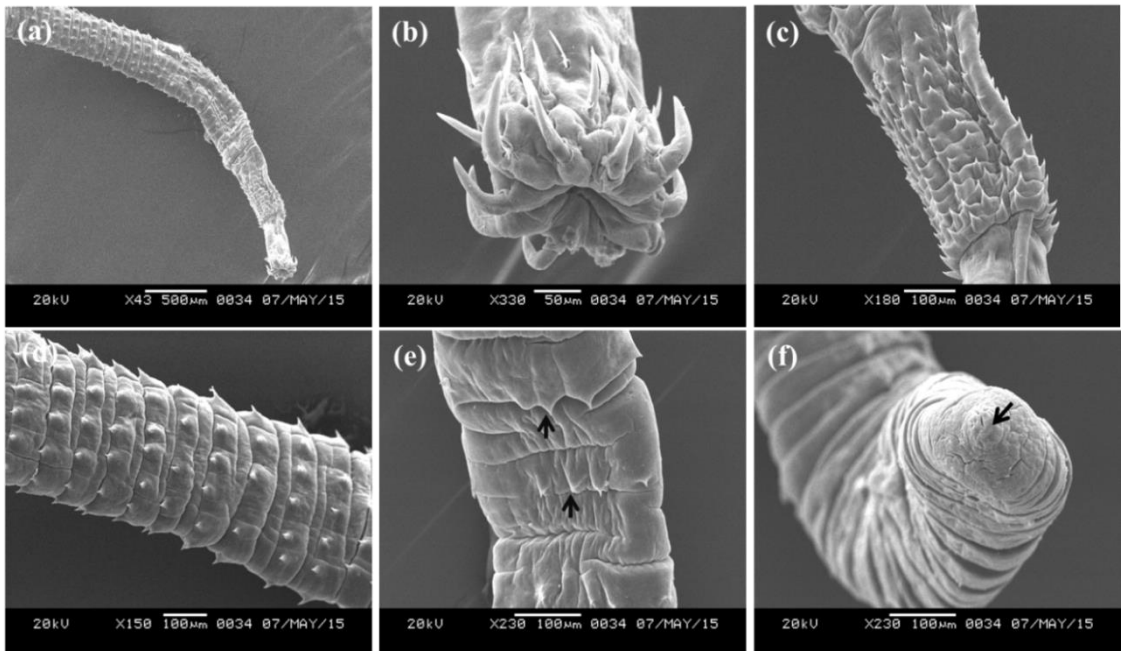

Fig. 2.16 Scanning electron microscopic (JSM 6360, JEOL) photographs of *Pallisentis ophiocephali* showing surface architecture

a) Anterior region of the worm

b) Enlarged view of the proboscis showing arrangement of hooks

c) Collar spines (backwardly directed) arranged in 13 closely set rings below the proboscis

d) Enlarged view of the mid body showing arrangement of backwardly directed spines

e) Enlarged view of posterior region of the body showing sparse spination and smooth surface

e) Enlarged view of tail end showing bursa

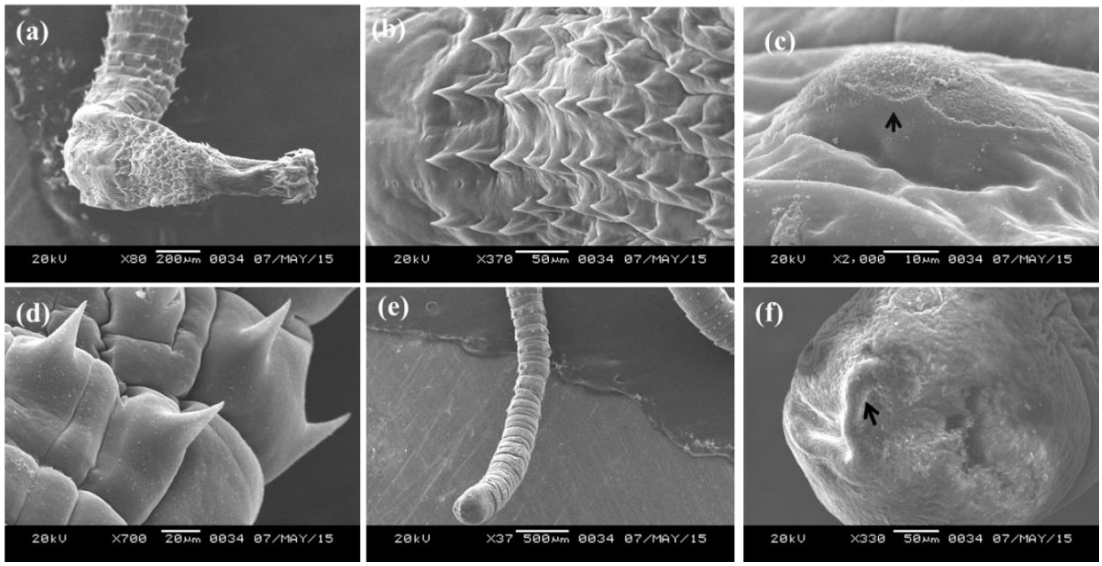

Fig. 2.17 Scanning electron microscopic (JSM 6360, JEOL) photographs of *Pallisentis indicus* showing surface architecture

a) Anterior end showing arrangement of spines and proboscis continuous with trunk

b) Collar spines arranged in longitudinal rows

c) Enlarged view of vulva (arrow)

e) Magnified view of body spine

f) Posterior region of body

f) Magnified view of mucron (arrow)

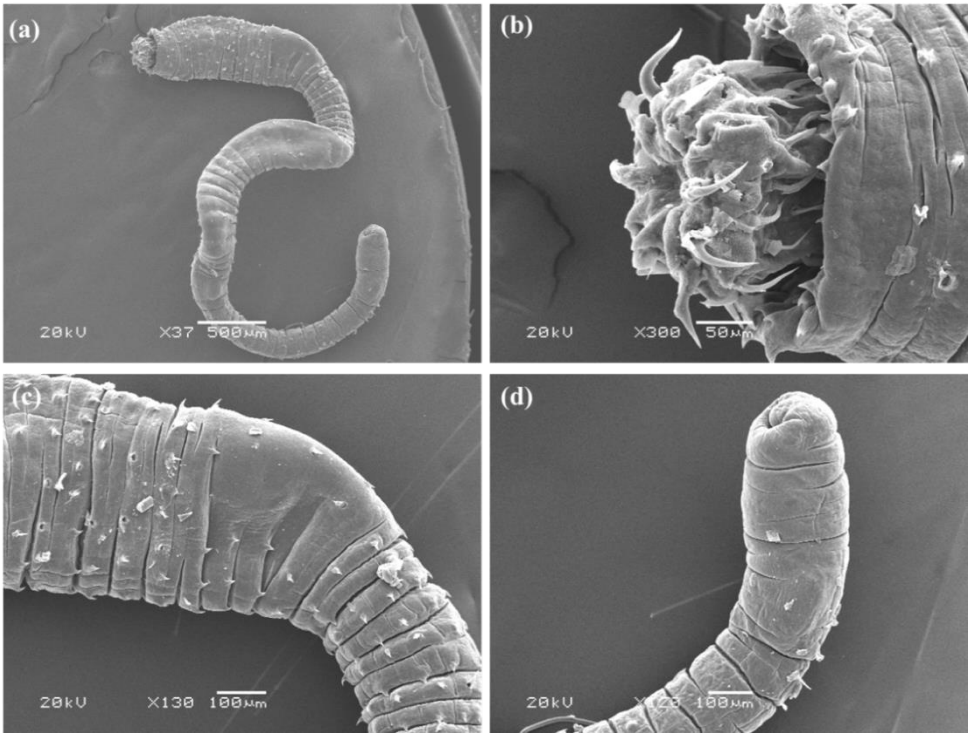

Fig. 2.18 Scanning electron microscopic (JSM 6360, JEOL) photographs of *Pallisentis sp.* showing surface architecture

a) General view of the whole worm

b) Enlarged view of proboscis

c) Mid-body showing arrangement of spines

d) Tail end showing smooth nature of the surface

2.4 Discussion

Among nematodes, *Paraquimperia manipurensis* showed an unique formation and arrangement of lip. The SEM images reveal that within the three lips there is division in each lip making it appear like six lipped structure which is usually three in other quimperids. The 10 pairs of caudal papillae are arranged in such a way that 4 pairs are pre-anal, 1 adanal and 5 pairs of post-anal (3 pairs sub-ventral, 2 pairs located laterally). Based on light microscopic studies, *Camallanus anabantis* and *Paracamallanus ophiocephali* were believed to be almost similar except in having large pharynx below cavity in *P. ophiocephali* but in our study, surface microtopographical study revealed that there is a difference in absence of sclerotized plate and amphids and presence of buccal teeth arranged in rows in *P. ophiocephali*. Moreover, the body cuticle is striated in *C. anabantis* whereas it is annulated in *P. ophiocephali* where the annules usually increase as it approaches the tail. Morphology of *Neocamallanus singhi* reported herein is almost similar to the redescription made by De and Majumdar (1984) however, little more details like the squarish shape of the head, cephalic papillae, orientation of the caudal papillae based on the SEM images has been illustrated in the present study. The numbers of caudal papillae recorded herein are 14 pairs (7 pairs of pre-cloacal papillae, 2 sessile cloacal papillae and 5 pairs of post-cloacal papillae) whereas 13 pairs were recorded by De and Majumdar (1984) (5 pairs of pre-cloacal papillae, 2 pairs of sessile cloacal papillae and 6 pairs of post-cloacal papillae). The difference in the number of the papillae may be due to the fact that the previous description was based solely on light microscopic study and thus could not be visible. This is the first report based on scanning electron microscopy on the nematodes *P. manipurrensis*, *N. singhi* and *P. ophiocephali*.

Scanning electron microscopic observation on Lytocestidae revealed considerable variation in the structure, dimensions and density of these surface extensions. In case of *Lytocestus attenuatus* the microtriches are filamentous whereas *L. filiformis* have scale-like tegument and that of *L. indicus* is rough, not scaly but *Djombangia penetrans* has a very smooth tegument. In adult *Senga lucknowensis*, scanning of the whole body surface showed that the tegument is scaly where each segment has sub-scales arranged transversely and directed posteriorly. The differences and diversity in the structure or pattern of microtriches are indicative of diversity in function like absorption, defense mechanism, attachment or locomotion (Kearn, 1988; Roy and Tandon, 2003).

Surface fine topographical observation on *Clinostomum philippinense* revealed the presence of an excretory pore surrounded by well-developed spines, thin thread like structure bisecting the ventral sucker internally, antero-lateral spination of the tegument and presence of genital pore in the ventral surface of the body which were found to be absent and/or different in other species such as *C. cuteneum* and *C. complantum* (Abidi *et al*., 1988; Gustinelli *et al*., 2010; Ngamniyom *et al*., 2012).

In the present study, the three species of *Pallisentis* found co-infecting the same host are *P. ophiocephali*, *P. indicus* and *Pallisentis* sp. where all the three species have a common major identifying character of the genus *Pallisentis* i.e., similar in having a proboscis with 4 circles of 10 hooks. However, formation arrangement and nature of smooth surface region are found to be little different which may not carry any taxonomic importance.

The present study illustrated for the first time the characteristic features of surface tegument and cuticle of helminth parasites occurring in different freshwater fishes of Manipur like *Neocamallanus singhi, Paraquimperia manipurensis, Senga lucknowensis*

and *Clinostomum philippinense*. Among nematodes though many features like alae (smooth or tuberculated surface), body cuticle (transverse or longitudinal striation or smooth surface), buccal capsule (compact or bifurcated lips, cephalic papillae, sclerotized plates, presence or absence of teeth), vulva (number of lips), anal papillae (number and arrangement of pre-anal, adanal and post-anal), mucron (single, double or triple in number) and spicule (shape, length, pointed, blunt, smooth or tuberculated) are common to all, their number, formation, orientation and distribution in different parts of the body revealed to be species specific. Similarly, in cestodes body tegument (smooth, tuberculated, ciliated, pappillaeted or spiny scale like pappilae) and scolex (smooth, spiny or specific type of folding) have diverse microtopographical features which are again revealed to be species specific. Trematode also showed species specific characteristic features as per their arrangement of different types of papillae and spines are concern, surrounding their oral and ventral sucker and a genital opening. Thus, SEM observations on the helminth parasites of fish provided an additional set of characters of taxonomic value to identify the parasites to species level.

CHAPTER 3
MOLECULAR CHARACTERIZATION OF HELMINTH PARASITES

3.1 Introduction

Identification of organisms is a rudimentary step to gaining a better knowledge and further investigation in the field of biology. In this context, morphological approach to identification is the most elementary and common method. However, an integrative method of both morphological and molecular studies has been widely accepted in the effective identification of an organism up to the species level, discriminating intra-specific variation and cryptic species (Sharma *et al*., 2016a; Janssen *et al*., 2017). Different molecular techniques have been used in systematics, phylogeny and diversity studies of helminth parasites by different workers (Olson and Tkach, 2005; Zhang *et al*., 2017).

Molecular approaches such as DNA based PCR methods have been largely used in understanding the phylogeny reconstruction using various genetic markers (Caira *et al*., 2013; Brabec *et al*., 2015). It has also proven useful in systematics solving the taxonomic status of parasites through DNA sequencing (Sahu *et al*., 2015; An *et al*., 2017). The species which are morphologically similar but genetically different could be discriminated and describing new species becomes more accurate using such techniques with genetic markers like nuclear ribosomal DNA (rDNA) and mitochondrial DNA (mtDNA) (Gustinelli 2010; Tandon *et al*., 2012 Locke *et al*., 2015).

The nuclear ribosomal DNA (rDNA) gene is a multigene family arranged in tandemly repeated clusters and each cluster contains a set of genes separated by spacer regions and it has been widely used in molecular taxonomy and phylogeny. Each rDNA

repeat of eukaryotes consists of external transcribed spacer (ETS), 18S or smaller subunit (SSU), 5.8S, and 28S or large subunit (LSU), which are highly conserved and are separated by variable regions of Internal Transcribed Spacer 1 (ITS1) and Internal Transcribed Spacer 2 (ITS2) (Long and Dawid, 1980). The nuclear rDNA gene markers have been favourably used, because it undergoes a rapid concerted evolution promoting intra-genomic homogeneity of the repeat unit. It is also highly variable even with closely related species and it has a highly conserved region. Thus, it has been used in various phylogenetic studies, identification and delineating different species, including helminth parasites (Dzikowski *et al*., 2004).

The rDNA ITS2 has been extensively used in resolving the taxonomic status and molecular phylogeny of helminth parasites, including parasites of fishes (Sereno-Uribe *et al*., 2013; Rosser *et al*., 2016). Because they have emerged as the locus of choice in answering questions related to taxonomy, population genetics, species identification and phylogenetic relationships of various helminth parasites (Athokpam and Tandon, 2014; Pekmezci *et al*., 2014; Sharma *et al*., 2016a; Lyngdoh *et al*., 2016). An additional advantage of using ITS2 is the possibility of predicting its secondary structure from the primary sequence data and is known to provide further information that can be useful in delineating closely related species (Coleman 2003, 2007). This approach has been successfully used in discriminating closely related species among plants, fungi and parasitic groups, including cestodes and trematodes (Prasad *et al*., 2011; Rampersad ,2014; Zhang *et al*., 2015).

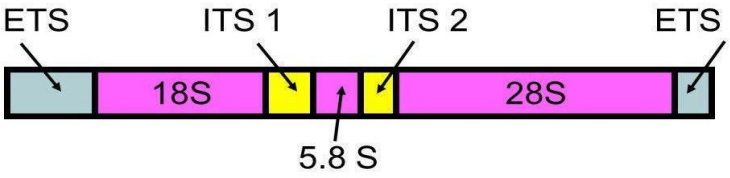

Fig. 3.1 Nuclear rDNA gene array with their associated spacers (ITS1 and ITS2)

(Source: http://images.slideplayer.com/16/4942872/slides/slide_44.jpg)

Studies have also been carried out on nuclear 18S rDNA (or small subunit – SSU) due to the highly conserved nature of the gene. It has been used to reconstruct phylogenetic branches at various taxonomic levels and resolves relationships of parasites above family level (Ndeda *et al*., 2013). It has a universal distribution and variable rate of evolution along the molecule; therefore, 18S has been profoundly studied to provide invaluable information regarding species identification and phylogenetic analysis among eukaryotes, including fish parasites (Kodedova *et al*., 2000; Oros *et al*., 2017.)

The mtDNA has a high rate of mutation which is faster than nuclear genome, therefore, it is effectively used for assessing evolutionary studies, genetic relationships as it can ascertain acquiring adequate nucleotide variation for comparing closely related organisms (Hassanin *et al*., 2013). Moreover, the substitution rate of mitochondrial protein is very low, allowing the amino acid changes accumulate slowly, thus supply details about genetic distances of related species (Taylor and Turnbull, 2005). The mitochondrial cytochrome oxidase subunit 1 (mtCO1) is simple and most conserved among the mtDNA genes, therefore it has been extensively used in molecular taxonomy and phylogenetics of various helminths including fish parasites (Athokpam and Tandon, 2014; Chai and Jung, 2017).

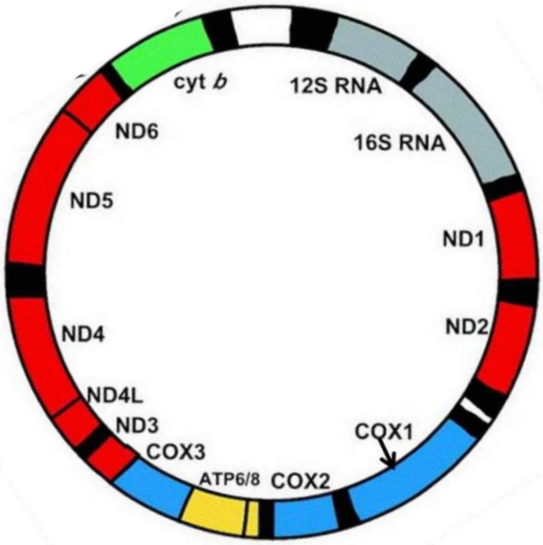

Fig. 3.2 Mitochondrial DNA showing cytochrome c oxidase subunit 1 (CO1)

(Source: https://ars.els-cdn.com/content/image/1-s2.0-S0047637401002883-gr1.jpg)

In the present study, nuclear and mitochondrial DNA gene markers were used to characterize and supplement morphology-based identification of the helminth parasites which could not be identified to species level through light microscopy.

3.2 Materials and method

Collection of Parasites

Parasites were collected as mentioned in the previous chapter. The collected parasites were washed thoroughly in PBS and fixed in 70% ethanol and preserved in -20°C for further molecular study. The helminth parasites which could not be identified up to species level based on morphology includes *Procamallanus* sp., *Posthodiplostomum* sp., *Clinostomum* sp., *Phyllodistomum* sp., *Senga* sp., *Pallisentis* sp., *Echinorhunchus* sp. and

a larval form of *Lytocestus* sp. Among these parasites, 5 of them, i.e., *Clinostomum* sp., *Phyllodistomum* sp., *Senga* sp., *Lytocestus* sp. and *Pallisentis* sp. were studied using the genetic markers CO1, ITS2, and 18S and they were successfully amplified. The other three parasites could not be undertaken for molecular methods because limited numbers of these parasites were recovered during the repetitive sampling and those few samples were used for morphological and morphometric studies.

DNA isolation

DNA extraction for the selected parasites were done using phenol chloroform-isoamyl method following standard protocol (Sambrook and Russell, 2001). The extraction procedure is given in the form of flowchart below.

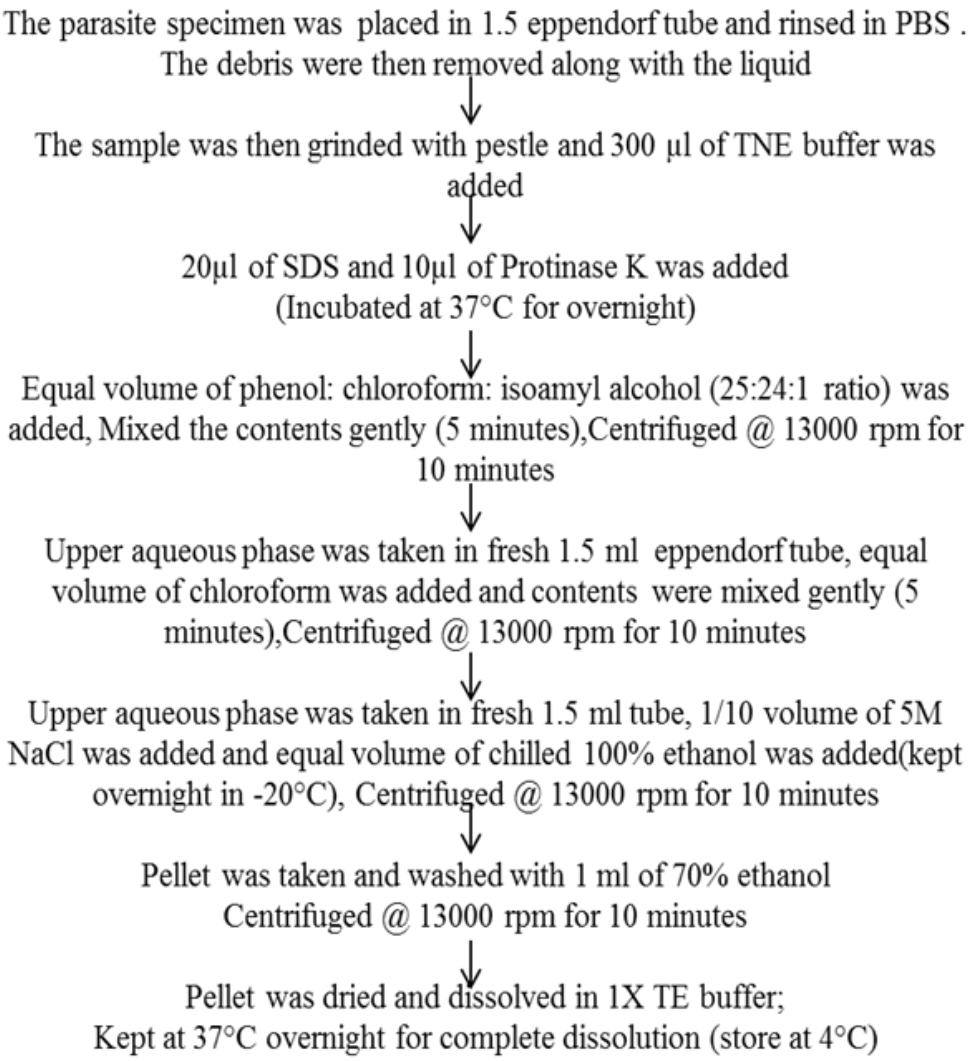

Fig. 3.3 Flowchart of the phenol-chloroform technique for DNA isolation (adopted from Sambrook *et al.*, 2001)

DNA amplification and Sequencing

The extracted DNA samples were used for PCR amplification. The genetic markers used in this procedure include rDNA 18S, ITS2 and mtCO1 regions.

Table 3.1 Details of the primers used

	Primer Sequences	References
ITS2:	3S (forward): 5'-GTACCGGTGGATCACTCGGCTCGTG-3'	Bowles *et al*., 1995
	A28 (reverse): 5'-GGGATCCTGGTTAGTTTCTTTTCCTCCGC-3'	
18S:	Worm A (forward): 5'CGAATGGCTCATTAAATCAG3'	Littlewood *et al*., 1999
	Worm B (reverse): 5'CTTGTTACGACTTTTACTTCC 3'	
CO1:	JB3 (forward): 5'-TTTTTTGGGCATCCTGAGGTTTAT-3'	Bowles and Mc Mannus, 1993
	JB4.5 (reverse): 5'-TAAAGAAAGAACATAATGAAAATG-3'	
	Dice1F (forward): 5'-TTWCNTTRGATCATAAG-3'	Moszczynska *et al*., 2009
	Dice14R (reverse): 5'-CCHACMRTAAACATATGATG-3'	Steenkiste *et al*., 2015

The PCR amplification was performed following standard protocol (White, 1993) with minor modifications (Sharma *et al*., 2016b).

Table 3.2 The PCR cocktail composition is as follows:

PCR cocktail		
DH_2O	-	13.5 µl
10X Taq Buffer	-	2.5 µl
dNTPs (mM)	-	2.5 µl
Primer 1 (20 pmol)	-	1 µl
Primer 2 (20 pmol)	-	1 µl
Taq Polymerase (3U/µl)	-	0.5 µl

$MgCl_2$	-	2.5 µl
Genomic DNA	-	2 µl
Total Volume		**25 µl**

Table 3.3 PCR thermal gradients of the genetic markers

	Temperature		
Gene markers	Denaturation	Annealing	Extension
ITS2	94^0C/45sec	55^0C/1min	72^0C/1min
18S	94^0C/1min	52^0C/1min	72^0C/2min
COX1	94^0C/1min	56^0C/1min	72^0C/1min

The amplified DNA products were then separated by electrophoresis through 1.6% (w/v) agarose gels in TAE buffer, stained with ethidium bromide, trans-illuminated under ultraviolet light and then photographed. The PCR products were purified using Genei Quick PCR purification kit followed by sequencing in both directions on an automated sequencer. The qualities of sequences generated were checked using sequence scanner software v2.0 and submitted to NCBI-GenBank and the accession numbers acquired. All the experiments were performed in the Department of Zoology, NEHU (except for sequencing, it was outsourced to Macrogen sequencing services, South Korea).

Sequence analysis

The generated sequences, along with sequences of the other related helminth species were retrieved from GenBank for analyses. The sequences were analyzed using various characterization tools and softwares, which are mentioned below.

Sequence scanner v 2.0

(http://www.appliedbiosystems.com/absite/us/en/home/support/softwarecommunity/free-ab-software.html)

The 2.0 version of sequence scanner software can be used to view, print and export sequence data generated by applied biosystem genetic analyzer. It generates graphically expressive reports on results, review all traces in thumbnail format and sort them by tracing quality and provides an instant toggle feature allowing to quickly switch between the details and thumbnail view.

BLAST (http://blast.ncbi.nlm.nih.gov/)

Basic Local Alignment Search Tool (BLAST) is a program for comparing primary biological sequence information (amino-acid sequences of different proteins – blastp/blastx or nucleotides of DNA sequences – blastn). The NCBI BLAST home page provides an access point for performing sequence alignment on the web. A BLAST search finds regions of similarity between biological sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance of matches.

DNA Baser Sequence Assembler v 3.5.3 (www.dnabaser.com/)

DNA Baser is a software for manual and automatic DNA sequence assembly, sequence analysis, file format conversion and contig editing. The reverse and forward sequences of rDNA 18S and 28S were combined in order to obtain longer sequences. This program was used to assemble multiple DNA samples or align to a reference sequence or groups of sequences by name.

ITS2 database webserver (http://its2.bioapps.biozentrum.uni-wuerzburg.de/)

The ITS2 database is a database for storage and retrieval of ITS2 sequence structures which also allows to process ITS2 sequences of our choice, annotate, predict the structure, detect motifs and BLAST search on the combined sequence structure information. The secondary structure of a sequence is predicted using an annotation profile Hidden Markov Models (HMMs) by identifying 25-nucleotide interaction of 5.8S rDNA subunit end with 25 nucleotides of the 28S rDNA (Eddy, 1998; Keller *et al*., 2009)

Multiple Sequence Alignment (MSA) using MUSCLE

(http://www.ebi.ac.uk/Tools/msa/muscle/)

MSA is an online tool for aligning three or more biological sequences, generally protein, DNA, or RNA. The output result of the Character can be used for inferring the intra and inter-specific relationship and phylogenetic analysis can be made to study the sequences. Visual depictions of the alignment illustrate mutation events such as point mutations (single amino acid or nucleotide changes) that appear as differing characters within a single alignment column, and insertion or deletion mutations (indels or gaps) that appear as "hyphens" in one or more of the sequences in the alignment.

BioEdit 7.2.5 (www.mbio.ncsu.edu/bioedit/bioedit.html)

Bioedit is sequence alignment editor and analysis software. It allows creating and manipulating alignment, run comparative analyses of nucleotide or amino-acid sequences from the edit window (Hall, 1999). In the present study it is used to generate a sequence identity matrix choosing sequence identity matrix option from the alignment menu and the genetic variation is calculated manually in percentage.

MEGA 6 (www.megasoftware.net/)

The software MEGA 6 (Tamura *et al*., 2013) was used for aligning the raw sequences. MEGA 6 is an integrated tool for performing automatic and manual sequence

alignment, constructing phylogenetic trees, web-based database mining, estimating rates of molecular evolution, inferring ancestral sequences, and testing best fit model of evolution. It has a sequence alignment program like MUSCLE and ClustalW amalgamated with it. The program MUSCLE was preferred to ClustalW for sequence alignment because of its accuracy and speed. This algorithm has three stages of drafts where it produces a rapid multiple alignment, a new accurate progressive alignment and refinement of the alignment produced.

Phylogenetic analysis

Phylogenetic trees were constructed for all the generated sequences and genetic relatedness of each taxon was inferred using the following bioinformatics tools.

ALTER (http://sing.ei.uvigo.es/ALTER/)

For generating various formats of files and converting one file to the other which are needed for running diffent phylogenetic programmes, ALTER was used (Glez-Pena *et al*., 2010). It is an online application tool to transform between different multiple sequence alignment formats. The working principle focuses on the specifications of mainstream alignment and analysis programme rather than on the conversion between more or less specific formats. It also allows identifying and removing identical sequences during the transformation process.

MrBayes v3.2.2 (http://mrbayes.sourceforge.net)

Phylogenetic trees for all the datasets were constructed to know the taxonomic status using Bayesian Inference (BI) in MrBayes (Ronquist *et al*., 2012). The Bayesian phylogenetic analysis uses the Markov Chain Monte Carlo (MCMC) methods to sample from the posterior probability distribution. By default, it uses Metropolis coupling to accelerate convergence with three heated and a cold chain running in parallel. Bayesian posterior probabilities were computed and the analysis was was run for 500000 generations and sampled every 1000 generations, with the first 25% of the trees being discarded as the 'burn-in' phase.

Figtree (http://tree.bio.ed.ac.uk/software/figtree/)

It is a graphical user-interface (GUI) application for viewing phylogenies where the input file is a tree file of various formats which can produce data in many formats. This application was used to view and edit the phylogenetic tree generated through MrBayes.

Secondary structure analysis

For prediction of secondary structure, ITS2 sequence was initially annotated using online tools ITS2 Database (Eddy, 1998) and then folded using the minimum folding energy module in RNAfold (Gruber *et al*., 2008).

RNAfold (http://rna.tbi.univie.ac.at/)

RNAfold is one of the core programs of the Vienna RNA package which can be used to predict the MFE (minimum free energy) of secondary structure of single sequences using the dynamic programming algorithm originally proposed by Zuker and Stiegler (1981). A single RNA or DNA sequence in plain text or FASTA format input can be pasted into the text box or uploaded as a file. By default, both the MFE and partition function (PF)

algorithm will be computed. The RNAfold server output contains the predicted MFE secondary structure in the usual dot-bracket notation.

3.3 RESULTS

The amplified sequences of *Clinostomum* sp. *Phyllodistomum* sp., *Senga* sp., *Lytocestus* sp. and *Pallisentis* sp. were submitted in NCBI Genbank and their accession numbers were generated. Primarily, all the submitted sequences were aligned with the sequences retrieved from Genbank. The generated sequences along with all the sequences of various species (a representative from each country or locality) necessary for the present study were retrieved from GenBank for analysis and comparison (Table 3.4, 3.5, 3.8, 3.9, 3.12, 3.14, 3.15). The molecular characterization of each aforementioned parasites and the phylogenetic analysis results are given below.

1. *Clinostomum* sp.

In the multiple sequence alignment of the CO1 gene, no gaps were observed but numerous mismatches were found. However, only a single mismatch was seen between *C. philippinense* of Thailand and isolate of the present study (Fig 3.4) which was also supported by the genetic similarity index analysis. Thus, indicating that our isolate is highly similar to *C. philippinense* of Thailand with very less genetic variation (Table 3.6). In case of ITS2, the multiple sequence alignment and sequence similarity index matrix analyses generated, revealed maximum homology with *Clinostomum* sp. of Nigeria and China and also with *C. tilapiae* of Nigeria and South Africa (Fig. 3.5; Table 3.7).

In order to corroborate the sequence analysis results, phylogenetic trees were constructed for the various species of *Clinostomum* for both the genes (CO1 and ITS2). For this, *Euclinostomum heterostomum* (KP721421 and KP721439) was taken as an out-group (Table 3.4 and 3.5). The CO1 phylogenetic tree was well resolved and the nodes were supported by high Bayesian posterior probability (Bpp) values. The CO1 inferred phylogeny depicted that the present species is closely related to *C. philippinense* (Thailand) which is supported by strong Bpp value of 98% (Fig. 3.6). However, in the ITS2 gene inferred phylogeny the species of query did not show any sort of association with any other species of *Clinostomum* erected separately (Fig. 3.7).

Annotation of the ITS2 sequence of the species in the present study revealed the length of ITS2 proper to be of 283bp. Folding of the primary transcript generated for the ITS2 secondary structure depicted the hallmark four helix model with branches, loops and bulges. The helix II showed the presence of pyrimidine-pyrimidine (U-U) mismatch whereas, the UGG and GGU motif was evident in helix III (Fig. 3.8).

Table 3.4 mtCO1 sequences of *Clinostomum* species used for sequence analysis and phylogenetic inference

Species	Accession No.	Locality	Host
1. *C. philippinense*	MF947448*	India	*Trichogaster fasciata*
2. *C. philippinense*	KP110523	Thailand	*Trichogaster microlepis*
3. *C. tilipiae*	KY649364	Nigeria	*Synodontis batensoda*
4. *C. phalacrocoracis*	KY906238	South Africa	*Clarias gariepinus*
5. *C. attenuatum*	KP150306	USA	*Lithobates pipiens*
6. *C.detruncatum*	KP110519	Brazil	*Synbranchus marmoratus*
7. *C. marginatum*	J JX630997	Mexico	*Ardea alba*
8. *C. complanatum*	KM923964	China	*Carassius auratus*
9. *C. tataxumui*	KJ504211	Middle America	*Tigrisom amexicanum*
10. *Euclinostomum heterostomum*	KP721421	Isreal	Cichlids

*Sequence generated for the study

Table 3.5 ITS2 sequences of *Clinostomum* species used for sequence analysis and phylogenetic inference

Species	Accession No.	Locality	Host
1. *Clinostomum sp.*	KX758630*	India	*Trigogaster fasciata*
2. *Clinostomum sp.*	KY865625	Nigeria	*Synodontis batensoda*
3. *C. tilipiae*	KX034048	S Africa	-
4. *C .tilipiae*	KY649353	Nigeria	*Synodontis batensoda*
5. *Clinostomum sp*.	KP110579	China	*Ctenopharyngodon idella*
6. *C. phalacrocoracis*	FJ609423	Kenya	*Ardea cinerea*
7. *C. cutaneum*	GQ339114	Kenya	*Ardea cinerea*
8. *C.complanatum*	JF718624	Italy	*Lepomis gibbosus*
9. *C. complanatum*	MF171131	Turkey	*Squalius cephalus*
10. *C. complanatum*	KF811010	India	*Heteropneustes fossilis*
11. *C. marginatum*	KU708007	USA	*Ardea alba*

12. *C. tataxumui*	KU156742	Middle America	*Tigrisoma mexicanum*
13. *Euclinostomum heterostomum*	KP721439	Isreal	Cichlids

*Sequence generated for the study

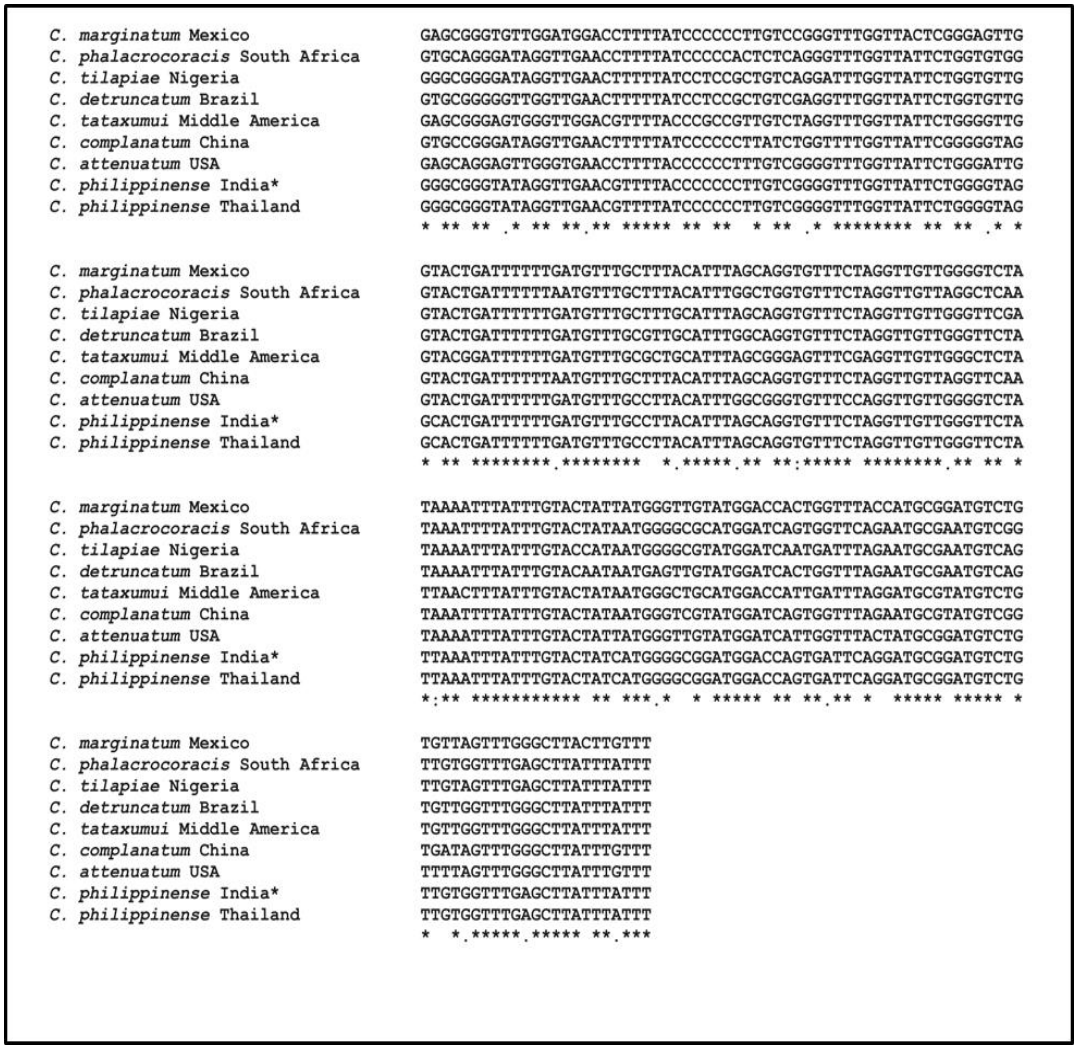

Fig. 3.4 Multiple sequence alignment of mtCO1 gene of *Clinostomum* sp. showing mismatches and complete alignment [Asterisks (*) just below the nucleotide base pairs shows well aligned sequences, whereas the space between the asterisks shows mismatches]

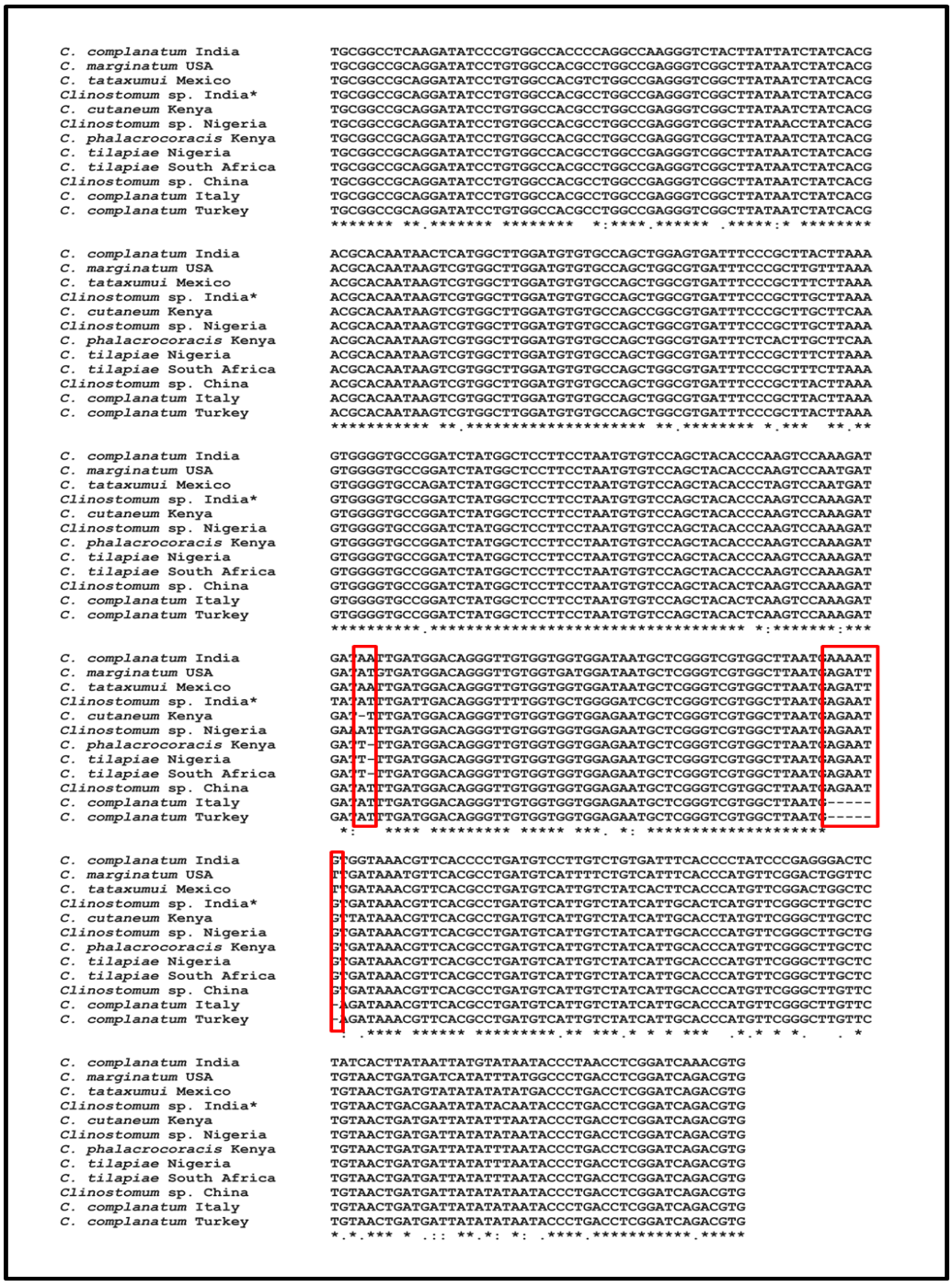

Fig. 3.5 Multiple sequence alignment of ITS2 gene of *Clinostomum* sp. showing gaps in highlighted boxes (red) and mismatches [Asterisks (*) just below the nucleotide base pairs shows well aligned sequences, whereas the space between the asterisks shows mismatches

Table 3.6 mtCO1 sequence similarity index matrix with values indicating % identities/ % differences among various isolates of species of *Clinostomum*

	1	2	3	4	5	6	7	8	9
1. *Clinostomum. philippinense**	**ID**								
2. *C. philippinense*	**99.5**/0.5	**ID**							
3. *C. tilapiae*	87.6/12.4	88.1	**ID**						
4. *C. phalacrocoracis*	86.2/13.8	86.6	88.6	**ID**					
5. *C. attenuatum*	85.2/14.8	84.7	82.7	82.7	**ID**				
6. *C. detruncatum*	83.7/16.3	84.2	90.6	85.7	86.2	**ID**			
7. *C. marginatum*	84.7/15.3	85.2	83.2	80.7	90.1	86.2	**ID**		
8. *C. complanatum*	85.2/17.5	85.7	87.1	89.1	83.7	85.7	85.7	**ID**	
9. *C. Tataxumui*	85.2/17.5	84.7	82.2	79.8	84.7	85.7	83.7	80.7	**ID**

[Note: The numbers in **bold** indicate the highest value; ID - identical]

Table 3.7 ITS2 sequence similarity index matrix with values indicating % identities/ % differences among various isolates of species of *Clinostomum*

	1	2	3	4	5	6	7	8	9	10	11	12
1. *Clinostomu*m sp. *	**ID**											
2. *Clinostomum* sp.	**95.9**/4.1	**ID**										
3. *C. tilapiae*	95.9/4.1	98.2	**ID**									
4. *C. tilapiae*	95.9/4.1	98.2	100	**ID**								
5. *Clinostomum* sp.	95.9/4.1	98.2	98.5	98.5	**ID**							
6. *C. phalacrocoracis*	95.3/4.7	97.6	98.8	98.8	97.6	**ID**						
7. *C. cutaneum*	95.0/5	97.3	98.5	98.5	97.3	98.5	**ID**					
8. *C. complanatum*	94.2/5.8	96.5	96.8	96.8	98.2	95.9	95.6	**ID**				
9. *C. complanatum*	94.2/5.8	96.5	96.8	96.8	98.2	95.9	95.6	100	**ID**			
10. *C. complanatum*	85.8/14.2	88.1	88.4	88.4	98.2	87.5	87.2	87.2	87.2	**ID**		
11. *C. marginatum*	91.6/8.4	93.6	94.2	94.2	98.2	93.6	93.3	93.0	93.0	86.4	**ID**	
12. *C. tataxumui*	92.4/7.6	94.2	94.7	94.7	98.2	93.6	93.3	93.3	93.3	87.2	94.7	**ID**

[Note: The numbers in **bold** indicate the highest value; ID - identical

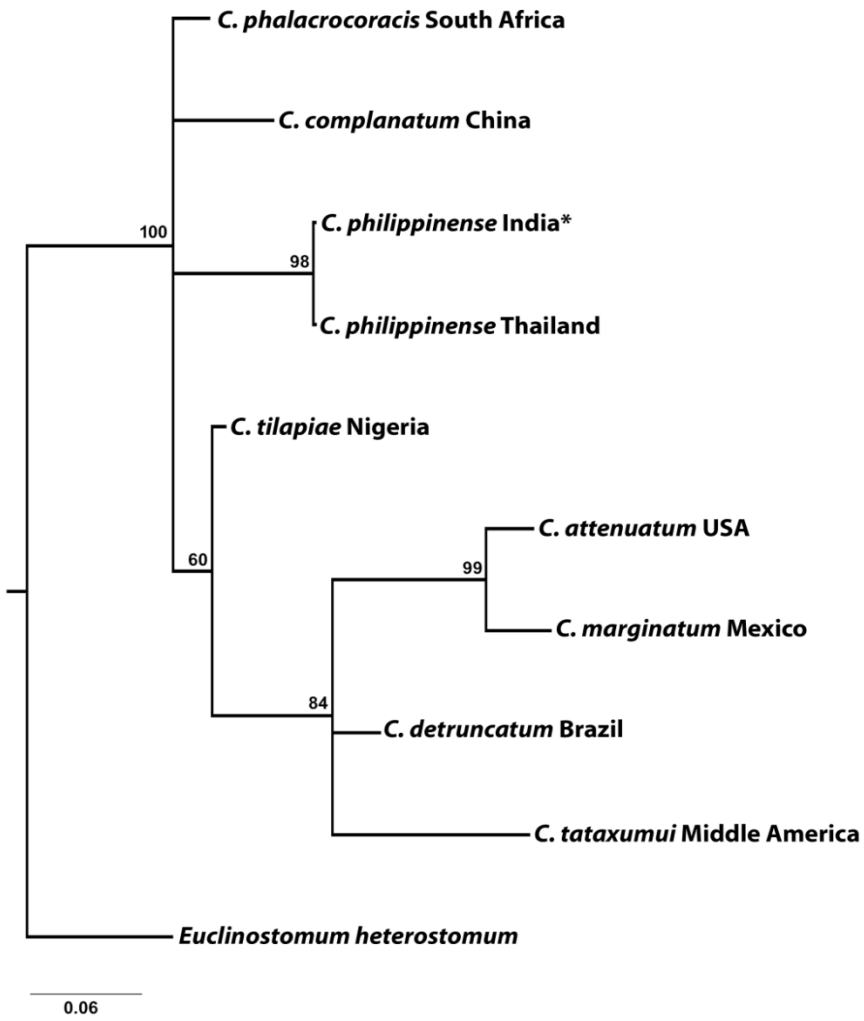

Fig. 3.6 Phylogenetic tree of *Clinostomum* species inferred via Bayesian Inference in MrBayes using mtCO1 gene regions. Numbers against the nodes indicate Bayesian posterior probability values

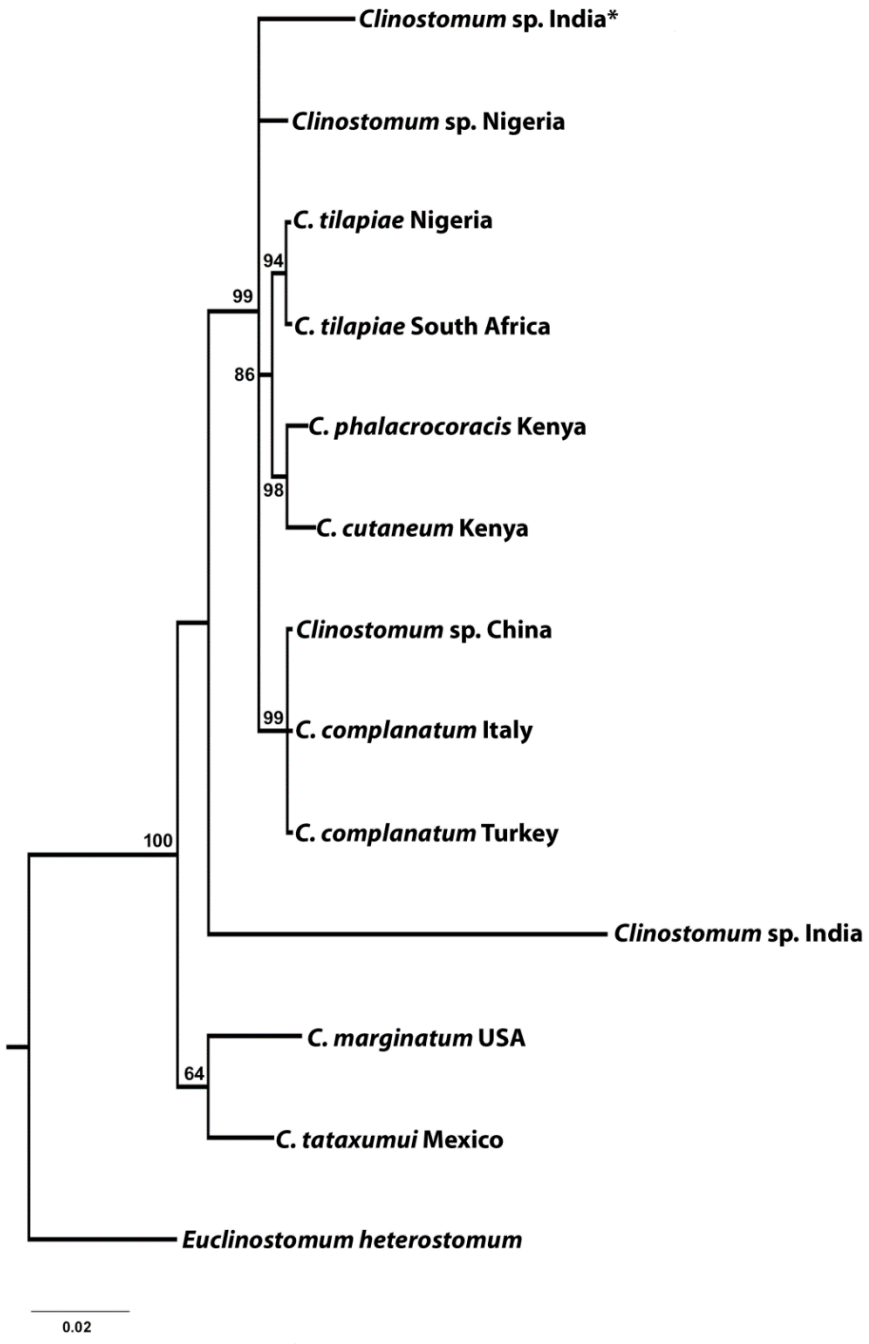

Fig. 3.7 Phylogenetic tree of *Clinostomum* species inferred via Bayesian Inference in MrBayes using rDNA-ITS2. Numbers against the nodes indicate Bayesian posterior probability values

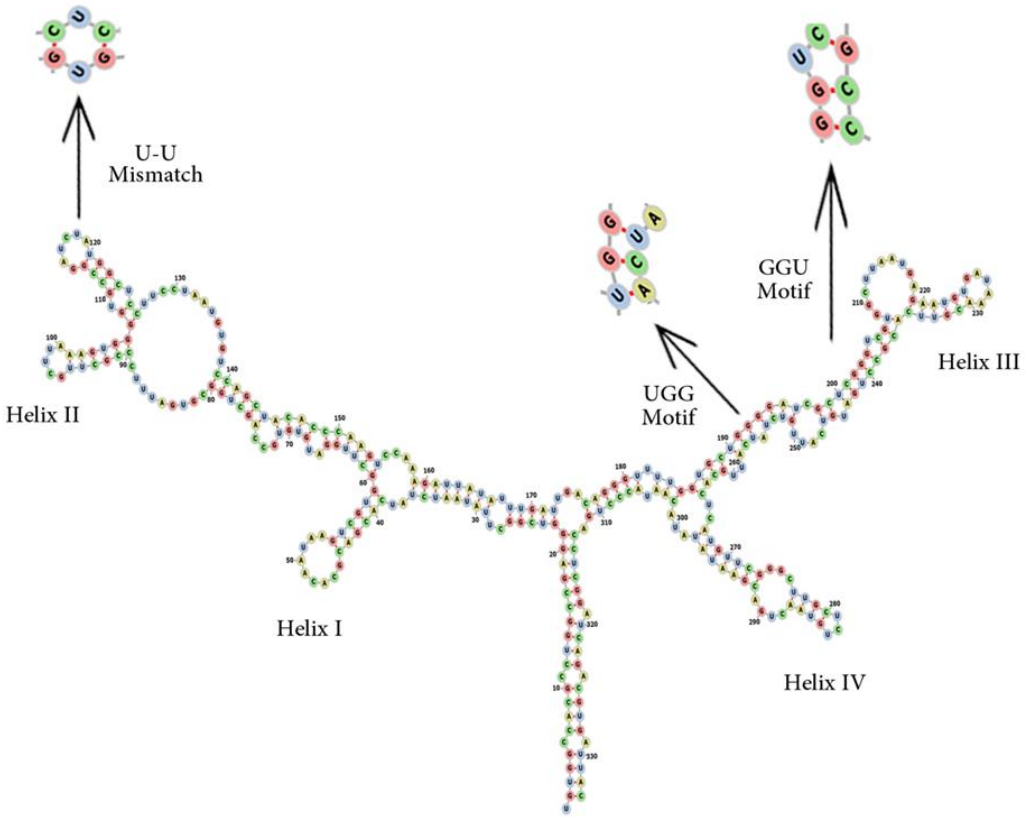

Fig. 3.8 Predicted secondary structure of the annotated ITS2 region of *Clinostomum* sp. based on centroid structure modeling generated via RNAfold taking the lowest negative minimum free energy structure (mfe = - 98.80 kcal/mol). Helices I to IV, U-U mismatch in helix II, UGG and GUU motifs in helix III are indicated

2. *Phyllodistomum* sp.

The multiple sequence alignment (MSA) of both the gene, i.e., CO1 and ITS2 showed several mismatches, however, the gaps are more prominent in alignment of ITS2 genes (Fig. 3.9 and 3.10). The similarity index study for both the gene revealed relatively low identity with other species. The maximum similarity of the present species was with *P. cribbi* for CO1 and *P. magnificum* for ITS2 (Table 3.10, 3.11).

For constructing a phylogenetic tree using region CO1 and ITS2, *Plagiorchis maculosus* (KJ533428 and KJ533391 repectively) was taken as the out-group (Table 3.8, 3.9). Both the phylogenetic trees were well resolved and the nodes were supported by Bpp values. The CO1 inferred tree showed our species is claded with with *P. cribbi* of Mexican isolate (Fig. 3.11) whereas the ITS2 phylogenetic tree showed the species of our study to be related to *P. magnificum* of Australia isolate (Fig. 3.12).

The predicted secondary structure for ITS2 region of *Phyllodistomum* sp. using centroid modeling generated via RNAfold revealed the presence of U-U mismatch in helix II, three motifs in helix III i.e., UGGU, GGU and UGG. The minimum free energy (MFE) of the generated structure is -113.10 kcal/mol (Fig. 3.13).

Table 3.8 mtCO1 sequences of *Phyllodistomum* species used for sequence analysis and phylogenetic inference

Species	Accession No.	Locality	Host
1. *Phylloditomum sp.*	MG948467*	India	*Channa gachua*
2. *Phylloditomum sp.*	AB987943	Japan	*Cyprinus carpio*
3. *P. cribbi*	KT376731	Mexico	*Zoogoneticus quitzeoensis*
4. *P. wallacei*	KT376726	Mexico	*Xenotaenia resolanae*
5. *P. brevicecum*	KC760183	Canada	*Umbra limi*
6. *P. inecoli*	KC760178	Mexico	*Heterandria bimaculata*
7. *P. parasiluri*	LC002524	Japan	*Silurus asotus*
8. *P. staffordi*	HQ325056	Canada	*Ameiurus melas*
9. *P. lacustri*	HQ325054	Mexico	*Ictalurus punctata*
10. *P. kanae*	AB979869	Japan	*Hynobius retardatus*
11. *P. centropomi*	KT376733	Mexico	*Centropomus parallelus*
12. *P. spinopapillatum*	KT376732	Mexico	*Profundulus balsanus*

13. *Plagiochis maculosus*	KJ533428	Czech Republic	*Lymnaea stagnalis*

*Sequence generated for the study

Table 3.9 ITS2 sequences of *Phyllodistomum* species used for sequence analysis and phylogenetic inference

Species	**Accession No.**	**Locality**	**Host**
1. *Phylloditomum sp.*	MG948466*	*India*	*Channa gachua*
2. *P. umblae*	KJ740509	Russia	*Coregonusalbula*
3. *P. magnificum*	KF013153	Australia	*Tandanus tantanus*
4. *P. folium*	KJ740504	Russia	*Abramis brama*
5. *P. angulatum*	KY307871	Russia	*Lota lota*
6. *P. macrocotyle*	KJ740520	Poland	*Dreissena polymorpha*
7. *P. pseudofolium*	KY307878	Russia	*Gymnocephalus cernuus*

8. *P. spinopapillatum*	KM659382	Mexico	*Profundulusbalsanus*
9. *P. Vaili*	KF013164	Australia	*Mulloidichthys vanicolensis*
10. *P. hoggetae*	KF013148	Australia	*Plectropomus leopardus*
11. *Plagiorchis maculosus*	KJ533391	Czech Republic	*Lymnea stagnalis*

*Sequence generated for the study

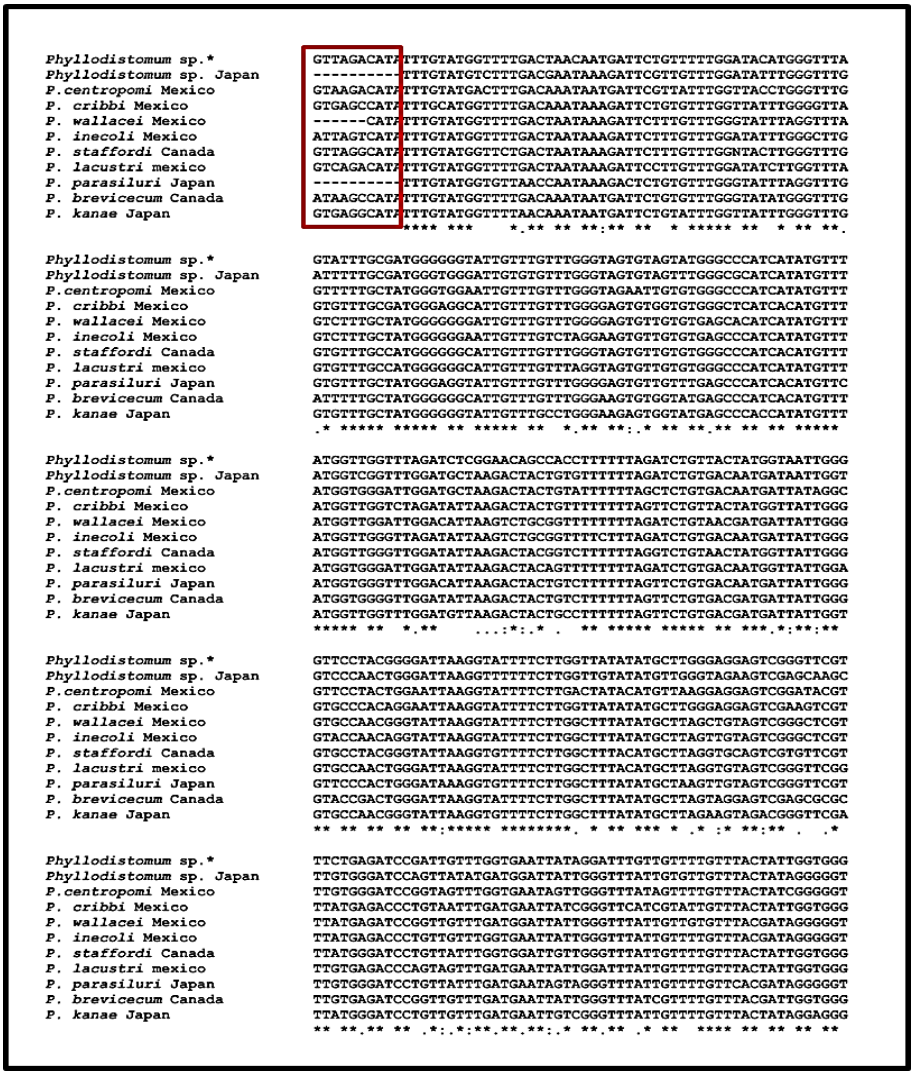

Fig. 3.9 Multiple sequence alignment of mtCO1 gene of *Phyllodistomum* sp. showing gaps in highlighted box (red) and mismatches [Asterisks (*) just below the nucleotide base pairs shows well aligned sequences whereas the space between the asterisks shows mismatches]

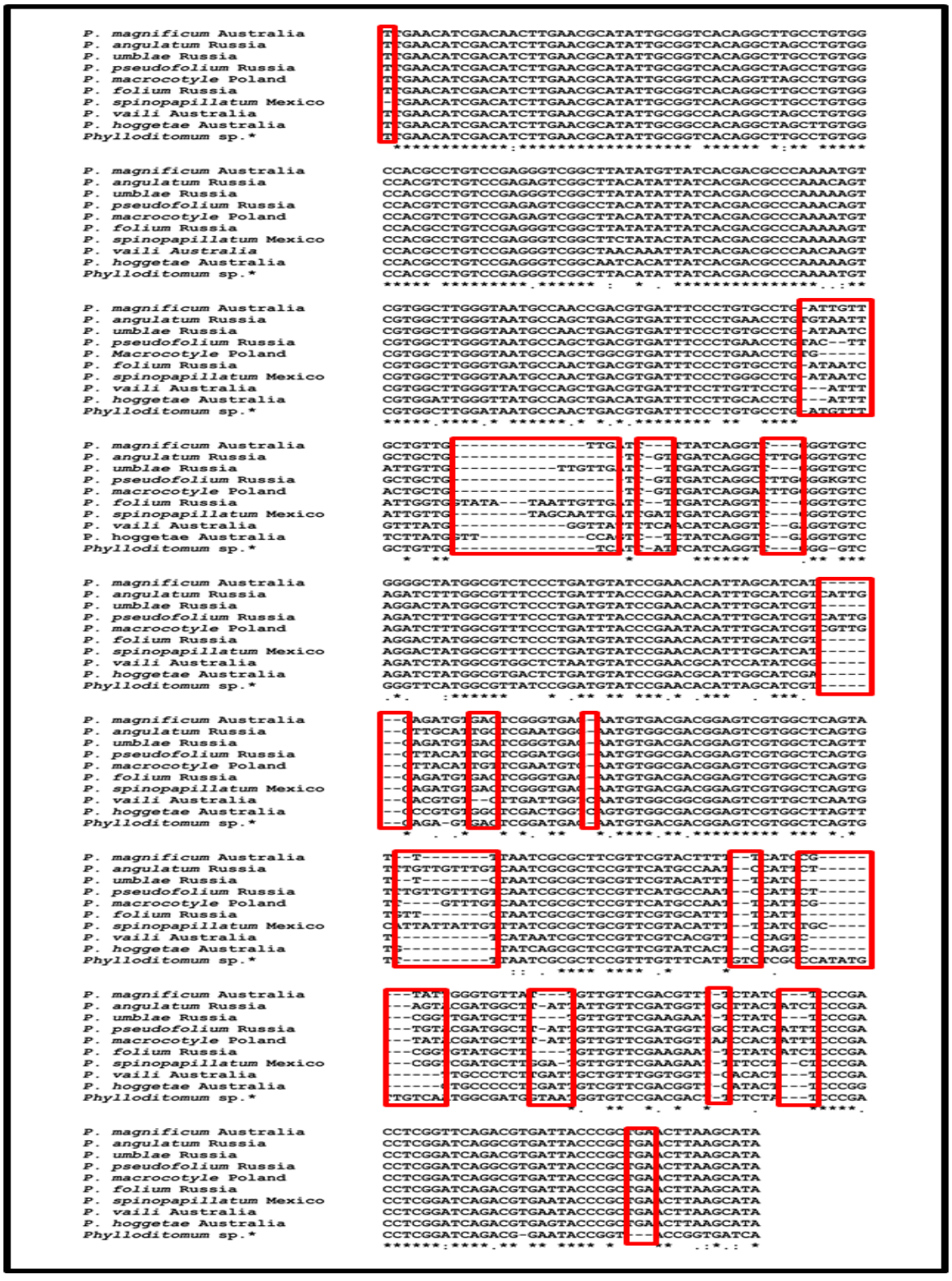

Fig. 3.10 Multiple sequence alignment of ITS2 gene of *Phyllodistomum* sp. showing gap (red) in highlighted boxes and mismatches [Asterisks (*) just below the nucleotide base pairs shows well aligned sequences whereas the space between the asterisks shows mismatches]

Table 3.10 mtCO1 sequence similarity index matrix with values indicating % identities/ % differences among various isolates of the species *Phyllodistomum*

	1	2	3	4	5	6	7	8	9	10	11
1. *Phyllodistomum* sp.*	ID										
2. *Phyllodistomum* sp.	75.0/25	ID									
3. *P. cribbi*	**82.9**/17.1	77.2	ID								
4. *P. wallacei*	79.5/20.5	80.4	81.1	ID							
5. *P. brevicecum*	81.4/18.6	80.1	84.7	84.4	ID						
6. *P. inecoli*	80.8/19.2	78.5	82.4	90.5	68	ID					
7. *P. parasiluri*	74.6/25.4	80.8	78.5	84.4	83.4	81.8	ID				
8. *P. staffordi*	82.7/17.3	77.5	84.7	85.3	85	86	82.5	ID			
9. *P. lacustri*	82.7/17.3	79.5	84.7	85.7	85.3	87	80.5	87.6	ID		
10. *P. kanae*	80.8/19.2	77.5	82.7	83.4	86.3	84.7	83.1	85.7	83.1	ID	
11. *P.centropomi*	79.2/20.8	79.5	78.8	78.8	82.1	80.5	78.2	81.8	83.4	81.4	ID

[Note: The numbers in **bold** indicate the highest value; ID - identical]

Table 3.11 ITS2 sequence similarity index matrix with values indicating % identities/ % differences among various isolates of the species *Phyllodistomum*

	1	2	3	4	5	6	7	8	9	10
1. *Phylloditomum* sp.*	ID									
2. P. umblae	77.1/22.9	ID								
3. *P. magnificum*	**77.9**/22.1	90.7	ID							
4. *P. folium*	74.5/25.5	94.9	86.7	ID						
5. *P. angulatum*	70.0/30.0	77.4	76.3	77.1	ID					
6. *P. macrocotyle*	69.0/31.0	77.3	77.1	76.3	92.1	ID				
7. *P. pseudofolium*	71.0/29.0	77.4	77.0	76.9	97.0	92.8	ID			
8. *P. spinopapillatum*	76.6/23.4	90.1	84.4	88.6	77.8	76.1	77.0	ID		
9. *P. vaili*	66.8/33.2	75.6	74.3	73.2	73.7	72.4	73.2	72.1	ID	
10. *P. hoggetae*	66.6/33.4	75.6	74.4	73.1	74.5	72.7	73.7	71.7	81.5	ID

[Note: The numbers in **bold** indicate the highest value; ID - identical]

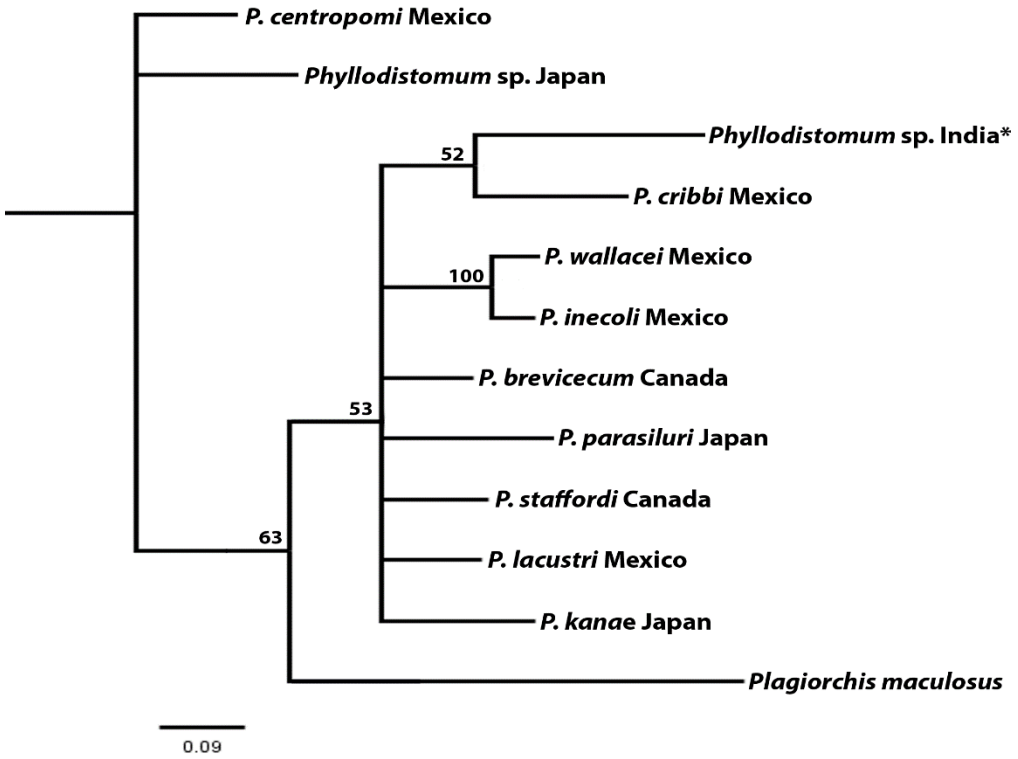

Fig. 3.11 Phylogenetic tree of *Phyllodistomum* species inferred via Bayesian Inference in MrBayes using mtCO1 gene regions. Numbers against the nodes indicate Bayesian posterior probability values

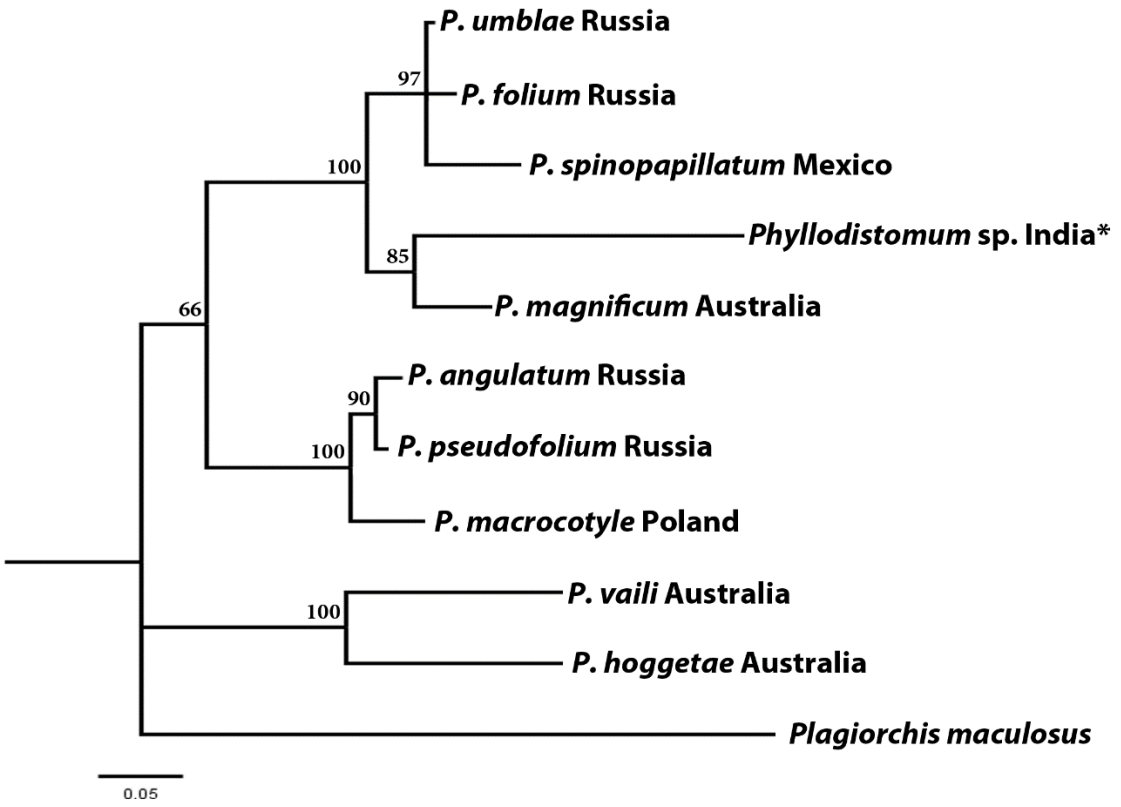

Fig. 3.12 Phylogenetic tree of *Phyllodistomum* species inferred via Bayesian Inference in MrBayes using ITS2 gene regions. Numbers against the nodes indicate Bayesian posterior probability values

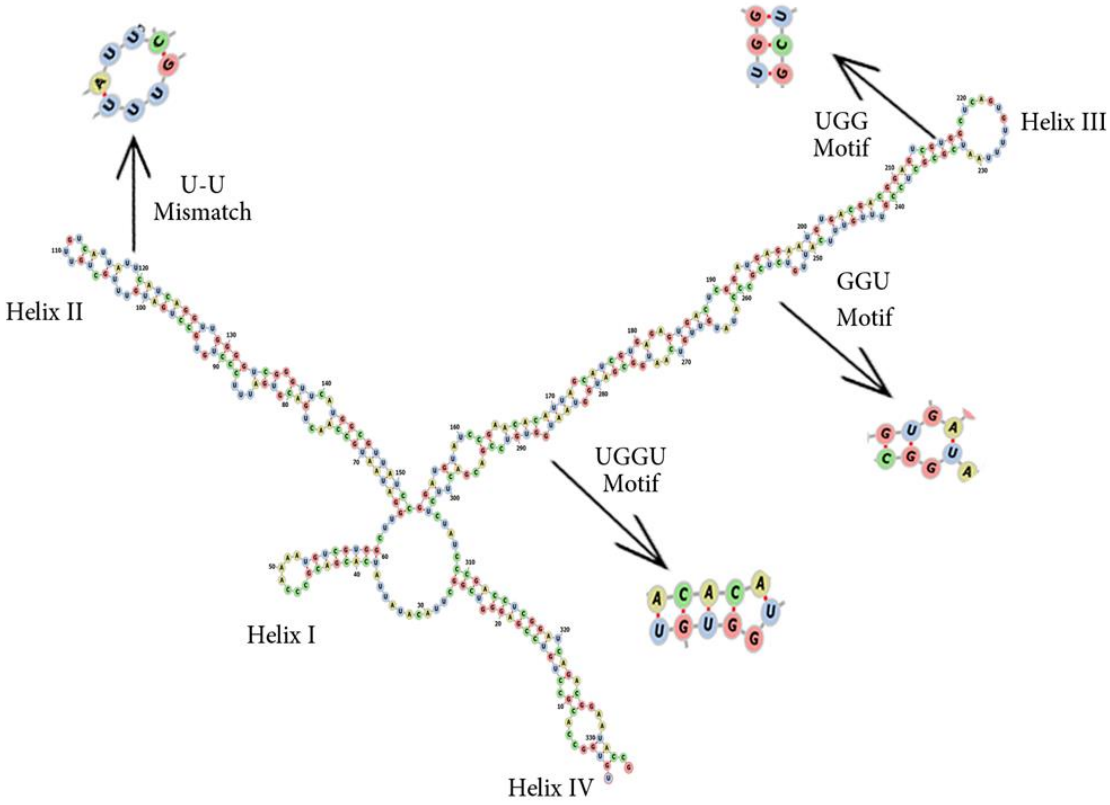

Fig. 3.13 Predicted secondary structure of the annotated ITS2 region of *Phyllodistomum* sp. based on centroid structure modeling generated via RNAfold taking the lowest negative minimum free energy structure (mfe = - 113.10 kcal/mol). Helices I to IV, U-U mismatch in helix II, UGGU, GGU and UGG motifs in helix III are indicated

3. *Lytocestus sp.*

The multiple sequence alignment of the 18S gene of *Lytocestus* taxa shows the presence of numerous gaps and mismatches (Fig. 3.14). The sequence similarity index matrix generated revealed maximum homology with *Lytocestus indicus* (Table 3.13) and the comparison of the genetic variation between our sequence with the rest revealed lowest in *Lytocestus indicus* and highest in *L. birmanicus* and *L. heteropneustii*. But the interspecific variation between *L. bimanicus* and *L. hereropneustii* is relatively low. Similar result was also depicted in the phylogenetic tree. *Djombangia penetrans* was taken as the outgroup (JQ034142) for constructing the phylogenetic tree**.** The Bayesian Inferred tree of 18S for the species of *Lytocestus* is well resolved and the nodes were supported by Bpp values. The 18S inferred phylogeny showed that the species collected from Manipur claded with *Lytocestus indicus*, of Indian isolate whereas *L. birmanicus* and *L. indicus* were erected separately with a long branch length (Fig. 3.15).

Table 3.12 18S sequence of *Lytocestus* species used for sequence analysis and phylogenetic inference

Sl. No.	Species	Accession No.	Locality	Host
1	*Lytocestus indicus*	KX758631*	India	*Clarias magur*
2	*L. indicus*	KC332243	India	*Clarias magur*
3	*L. birmanicus*	KC332244	India	*Clarias magur*
4	*L. heteropneustii*	KC332245	India	*Heteropneustus fosillis*

5.	*Djombangia penetrans*	JQ034142	India	*Clarias magur*

*Sequence generated for the study

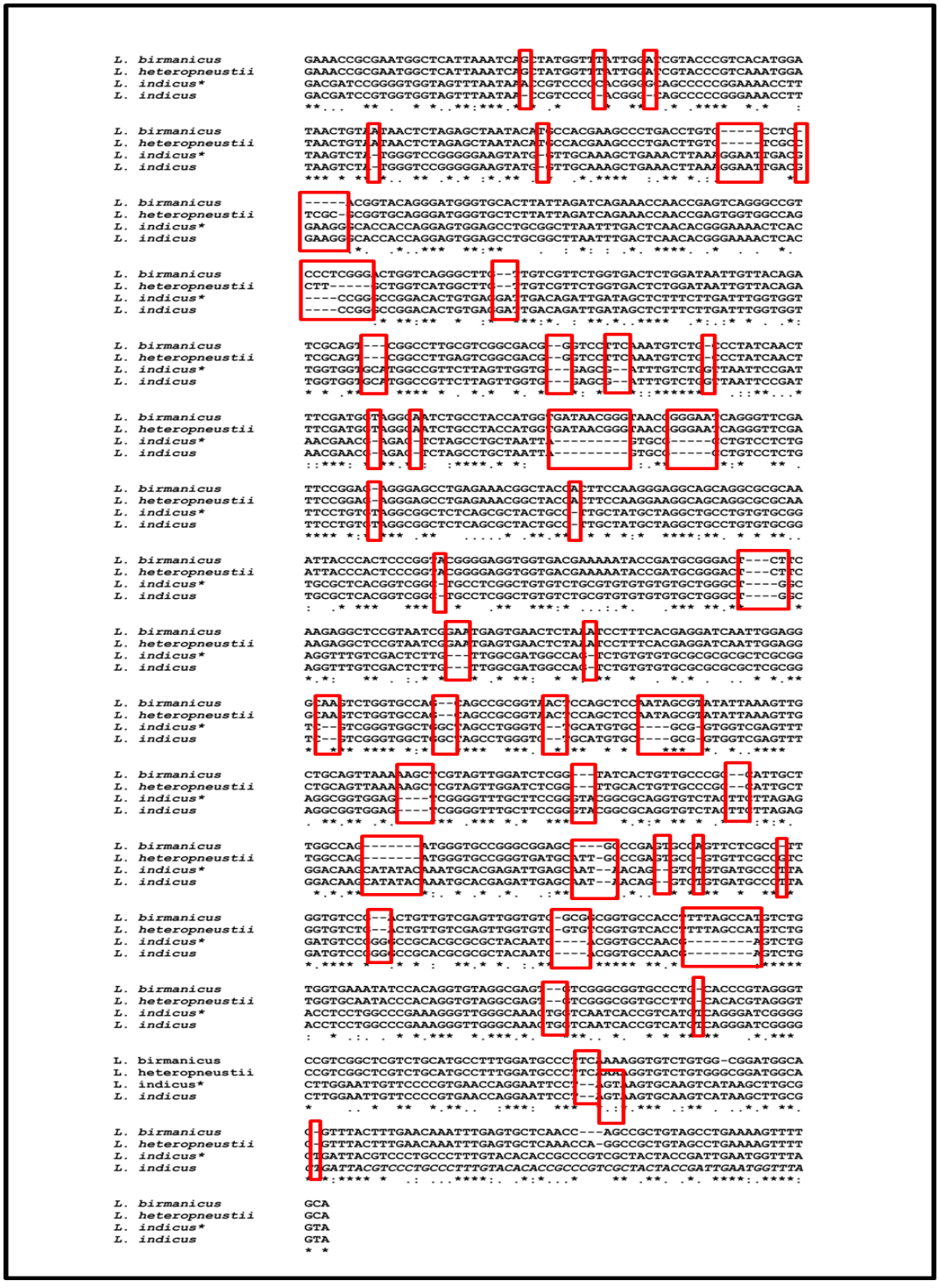

Fig. 3.14 Multiple sequence alignment of 18S gene of *Lytocestus* sp. showing gap in highlighted (red) boxes and mismatches [Asterisks (*) just below the nucleotide base pairs shows well aligned sequences whereas the space between the asterisks shows mismatches]

Table 3.13 18S sequence similarity index matrix for the various species of *Lytocestus*

	1	2	3	4
1. *L. indicus**	ID			
2. *L. indicus*	**99.4**/0.6	ID		
3. *L. birmanicus*	40.0/60	40.0	ID	
4. *L. heteropnuestii*	40.7/60	40.7	94.1/5.9	ID

[Note: The numbers in **bold** indicate the highest value; ID - identical]

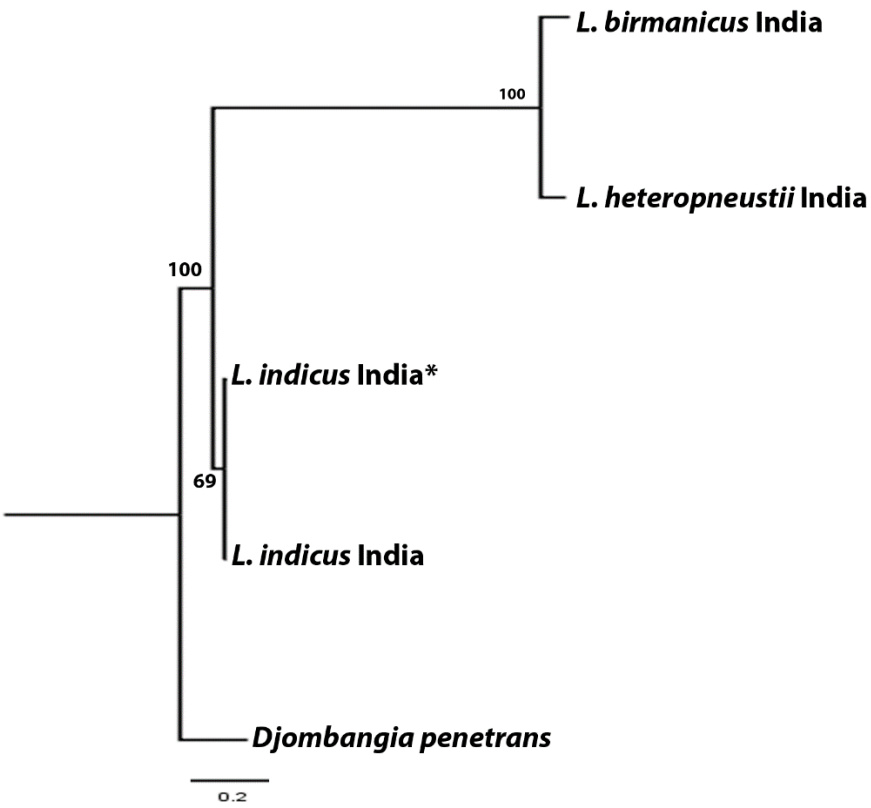

Fig. 3.15 Phylogenetic tree of *Lytocestus* species inferred via Bayesian Inference in MrBayes using 18S gene regions. Numbers against the nodes indicate Bayesian posterior probability values

4. *Senga* sp.

The multiple sequence alignment showed gaps in three sites in sequence of our species *S. lucknowensis* (Fig. 3.16). The similarity index matrix revealed that the sequence of our species to be highly identical to *Senga lucknowensis* of Vietnamese isolate and *Senga vishakapatnamensis* of Indian isolate (Table 3.15). The variation among them is almost negligible, i.e., 0.1%. This similarity between the species is also observed in the phylogenetic tree. The Bayesian inferred phylogenetic tree is well resolved with 98% Bpp values. The tree depicted that our isolate, *Senga lucknowensis* of Vietnamese isolate and *Senga vishakapatnamensis* of Indian isolate formed a separate clade just as the similarity index result had depicted (Fig. 3.17).

Table 3.14 18S sequences of *Senga* species used for sequence analysis and phylogenetic inference

Species	Accession No.	Locality	Host
1. *Senga lucknowensis*	KU761847*	India	*Channa punctata*
2. *S. lucknowensis*	KR780938	Vietnam	*Mastacembelus armatus*
3. *S. vishakapatnamensis*	KR780937	India	*Channa punctata*
4. *S. magna*	KR780960	Russia	*Siniperca chautsi*
5. *Bothriocephalus cuspidatus*	KR780955	USA	*Sander vitreus*

*Sequence generated for the study

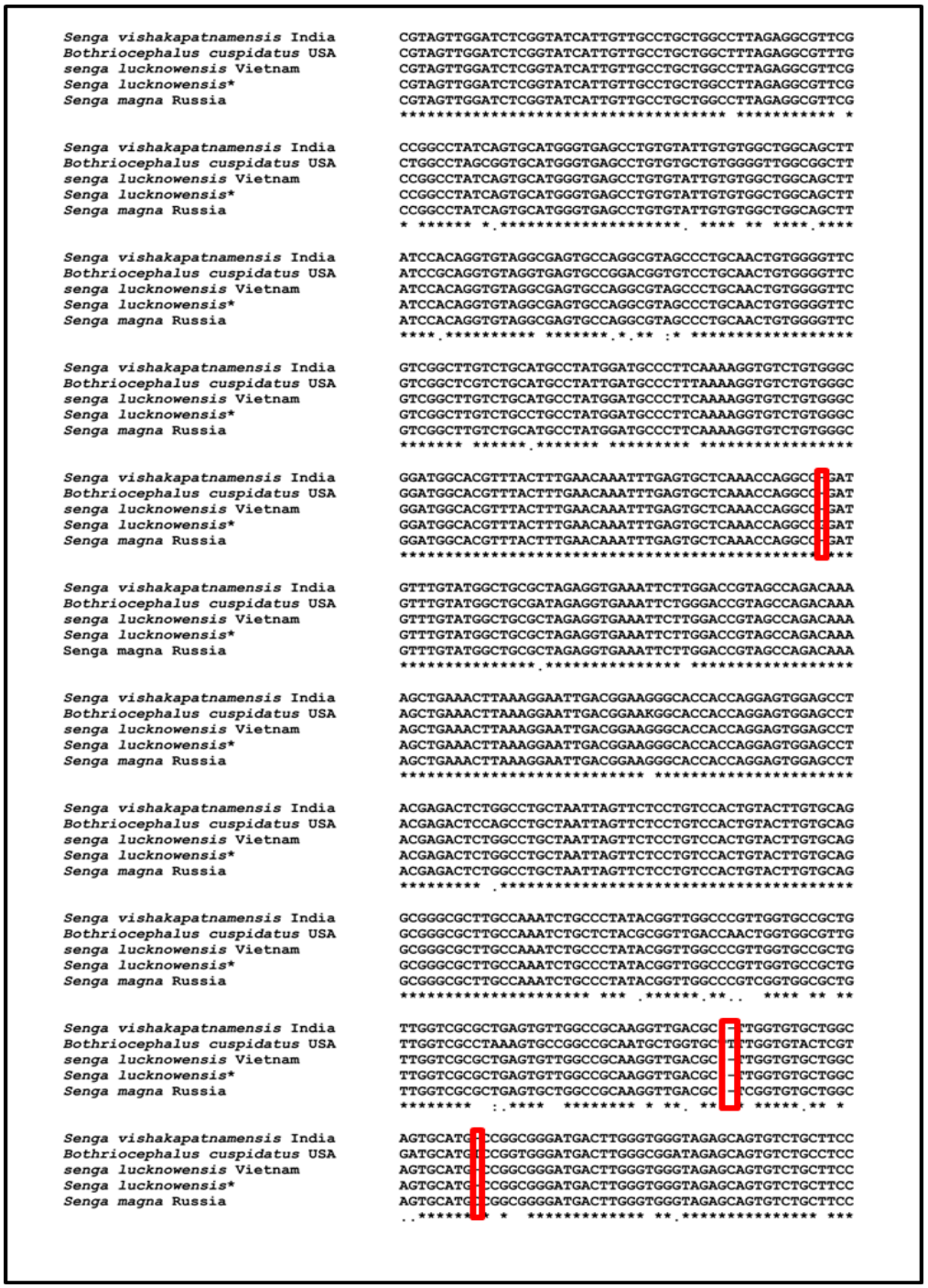

Fig. 3.16 Multiple sequence alignment of 18S gene of *Senga sp.* showing gap (red) in highlighted boxes and mismatches [Asterisks (*) just below the nucleotide base pairs shows well aligned sequences whereas the space between the asterisk shows mismatches]

Table 3.15 18S sequence similarity index matrix for the various species of *Senga*

	1	2	3	4	5
1. *Senga lucknowensis* *	ID				
2. *S.lucknowensis*	**99.8**/0.2	ID			
3. *S. vishakapatnamensis*	99.7/0.3	10	ID		
4. *S. magna*	99.4/0.6	99.5	99.5	ID	
5. *Bothriocephalus cuspidatus*	96.5/3.5	96.6	96.6	96.5	ID

[Note: The numbers in **bold** indicate the highest value; ID - identical]

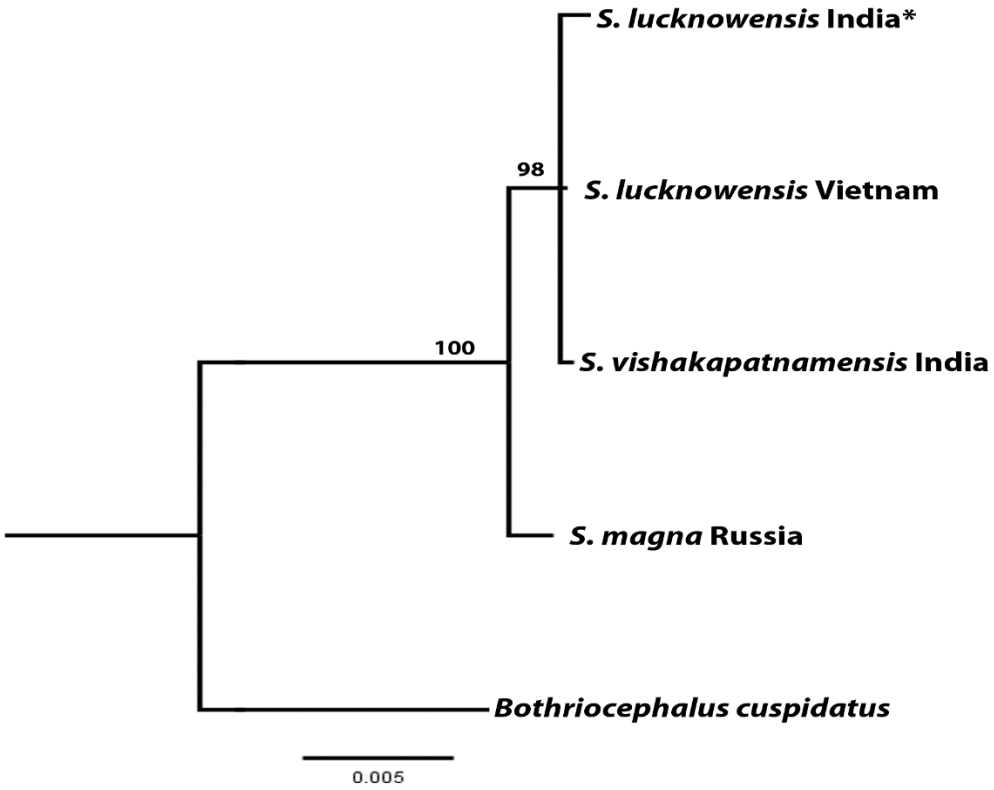

Fig. 3.17 Phylogenetic tree of *Senga* species inferred via Bayesian Inference in MrBayes using 18S gene regions. Numbers against the nodes indicate Bayesian posterior probability values

5. *Pallisentis* sp.

For sequence analysis and comparison of the 18S region of *Pallisenti*s sp., different genus of the taxa Eoacanthocephala from BLAST result were chosen since only one member of the genus of *Pallisentis* was available in Genbank for this particular gene. Therefore, the necessary sequences retrieved from the Genbank along with the sequence generated for the present study was considered for analysis and phylogenetic Inference (Table 3.16).

The multiple sequence alignment result showed many gaps and mismatches in the aligned sequence (Fig. 3.18). The similarity index matrix revealed the species query to have maximum homology with *Pallisentis* sp. of Indian isolate from the host *channa punctata* with genetic variation of about 0.4% (Table 3.17). The phylogenetic tree inferred via Bayesian inference supported the result of similarity index generating well resolved tree where species of the present study claded with *Pallisentis* sp. with nodal support Bpp values of 100% (Fig. 3.19).

Table 3.16 18S sequences of taxa Eoacanthocephala used for sequence analysis and phylogenetic inference

Sl. no.	Species	Accession No.	Locality	Host
1	*Pallisentis sp.*	MF437351*	India	*Channa Striata*
2	*Pallisentis sp.*	KU715089	India	*Channa punctata*
3	*Neoechinorhynchus pseudemydes*	KU363973	Iran	*Capoeta aculeata*
4	*Floridosendis mugilis*	AF064811	Mexico	-
4	*Acanthosentis sp.*	KY305530	India	-

6	*Moniliformis moniliformis*	HQ536017	Spain	*Rattus rattus*

*Sequence generated for the study

Fig. 3.18 Multiple sequence alignment of 18S gene of taxa Eoacanthocephala showing gaps (red) in highlighted boxes and mismatches [Asterisk (*) just below the nucleotide base pairs shows well aligned sequences whereas the space between the asterisk shows mismatches]

Table 3.17 18S sequence similarity index matrix for the various species of taxa Eoacanthocephala

	1	2	3	4	5
1. *Pallisentis sp.**	ID				
2. *Pallisentis sp.*	**99.6**/0.4	ID			
3. *Neoechinorhynchus pseudemydes*	89.3/10.7	89.3	ID		
4. *Floridosendis mugilis*	87.6/12.4	86.7	93.2	ID	
5. *Acanthosentis sp.*	86.9/13.1	86.9	87.7	86.9	ID

[Note: The numbers in **bold** indicate the highest value; ID - identical]

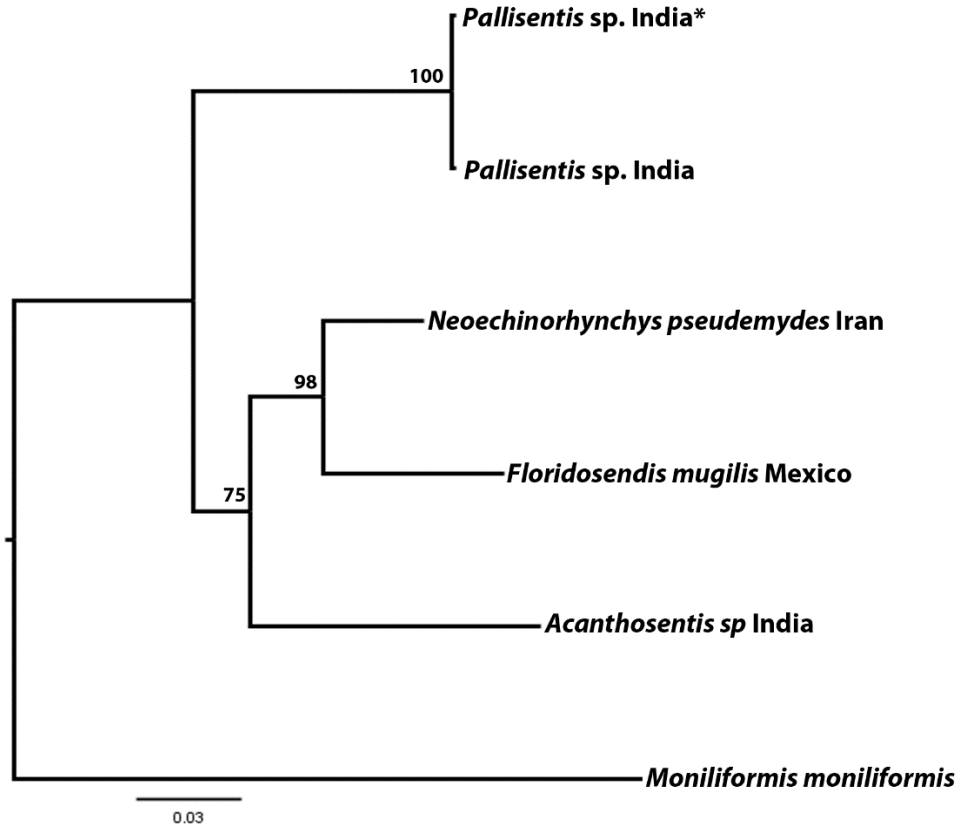

Fig. 3.19 Phylogenetic tree of the taxa Eoacanthocephala inferred via Bayesian Inference in MrBayes using 18S gene regions. Numbers against the nodes indicate Bayesian posterior probability values

3.4 Discussion

Among the five helminth parasites subjected to molecular characterization, it is revealed that for *Clinostomum* sp. comparison the multiple sequence alignment (MSA) and similarity index for the gene region CO1 and ITS2 showed that CO1 gives a better result. In the ITS2 sequence, the maximum homology is 95.9% to *Clinostomum* sp. whereas in case of CO1 it is identical up to 99.5% with *Clinostomum philippinense*. Similar types of results were also observed in the phylogenetic trees, i.e., in ITS2 inferred tree, our isolate did not cluster in a separate clade with any putative species of *Clinostomum*. However, in CO1 inferred tree our species claded with *Clinostomum philippinense*. In India, the *Clinostomum* species of common occurrence is represented by *C. complanatum* (Shareef and Abidi, 2012; Rizvi *et al*., 2012) which is distinctly different in morphological and molecular features from the one we studied. Molecular analysis of different parameters in the present study clearly indicates that the species under study belongs to *Clinostomum philippinense*. The ITS2 secondary structure information of our species is provided for characterization, but the comparison with other specimen of *C. philippinense* was not possible since data sequenced for this particular species was not available in Genbank for ITS2 region. This is the first report of *C. philippinense* from India. Though, the species is reported from Thailand (Yooyen *et al*., 2006).

Phyllodistomum is one of the largest genera comprising many species among trematodes (Zhokhov and Zoxob, 2010; Nakao, 2015). The review of literature of this genus reveals many synonymies among the species thus taxonomic status of many species is quite chaotic. Therefore, molecular analyses carried out in our present study showed that the variation between the species studied and others is very high. The results showed that the metacercaria of our study belongs to *Phyllodistomum* sp. When compared with the other species from India, it showed some similarity in morphology with *P. chauhani* but has a significant difference in excretory pore where it is terminal moreover, the only molecular data available for this genus from India is of 28S rDNA gene and therefore could not be compared (Chaudhary *et al*., 2016). Species level identification for this digenetic trematode was not possible because the high variation seen in the results suggested that it could be any of the species which has not been sequenced or it could be undescribed species. Since, what we have sequenced is not conspecific with other specimen of CO1 and ITS2 available in the Genbank.

The adult form of Lytocestidae could be identified through morphological studies and many species have been identified using such methods since the reproductive organs are well developed in adults (Tandon *et al*., 2005; Bhure *et al*., 2010). But, larval stages are not distinguishable up to species level by studying their morphological features. During our collection, metacestodes were sampled and molecular and bioinformatic tools were used to specify its taxonomic position. The molecular information gathered in our study revealed that there is a high inter-specific variation. The similarity index matrix showed that the species of our study is 99.4% identical to *Lytocestus indicus* of Indian isolate. The similar result is inferred in phylogenetic tree where our species is claded with

L. indicus with nodal support of 100% Bpp values. Therefore, the species sequenced confirmed the present species as *L. indicus*.

The 18S sequence generated from *Senga* sp. was first checked in BLAST, NCBI and it matched with the taxa *Senga* where the result follows in accordance with the classification of Bothriocephalidae based on morphological traits (Kuchta *et al*., 2008). The molecular analysis of the sequence query showed an interesting result. It was observed that the inter-specific variation among *Senga* is very less which means they are very similar to one another. The similarity index matrix showed that our *Senga* sp. is identical to *S. lucknowensis* of Vietnamese isolate (99.8%) and well as *S. vishakapatnamensis* (99.7%) with a variation between them in just 0.1. Moreover, in the phylogenetic tree, all the three species were clustered together in one clade with bpp value. The slight difference could be because of the geographical variation. It has clearly indicated from all the molecular information that the species of our study belongs to *Senga lucknowensis*.

Sequence analysis and phylogenetic tree inferred for acanthocephalan parasite, *Pallisentis* sp. depicted the maximum homology with *Pallisentis* sp. of Indian isolate with a similarity index of 99.6%. A similar type of result is seen in the phylogenetic tree, where it revealed that our species nested separately with *Pallisentis* sp. Comparison with other species of *Pallisentis* was not possible because only *Pallisentis* sp. sequences were available in Genbank for this gene region. Thus, it could not be identified up to species level. Therefore, the parasite was identified up to genus level and it belongs to the member of the taxa *Pallisentis*.

The present study provides the molecular characterization and identification of 5 helminth parasites viz., *Clinostomum philippinense*, *Phyllodistomum* sp., *Lytocestus indicus, Senga lucknowensis* and *Pallisentis* sp. The discription of ITS2 secondary morphometric information of *C. philippinense* and *Phyllodistomum* sp., which otherwise is lacking have also been added which in future can be used as valuable information for comparative studies.

The present study also proves the benefit and utility of molecular tools in delineating and identification of parasite having medicoveterinary importance.

CHAPTER 4
SEASONAL VARIATION IN PREVALENCE, ABUNDANCE AND MEAN INTENSITY OF HELMINTH PARASITES

4.1 Introduction

In the food industry, aquaculture plays an important role in the economy and it is one of the fastest growing sectors where, the fish meat is in high demand because of its nutritive values (Tidwell and Allan, 2001). Nonetheless, the safety concerns related to fish consumption due to the presence of parasites persist (Ljubojevic *et al*., 2015). Parasite infections can also cause harm to their hosts through changes in the hosts' physiology, behaviour, nutritional disturbances and injuries. Moreover, fatal secondary infections could occur because of severe weakness caused by their infections (Panayotova-Pencheva, 2013; Rondon *et al*., 2017).

In ecological studies of the host, the helminth community study is considered to be one of the important components, as changes in its structure can reflect environmental disturbances and also be used as indicators of an environment (Kvach *et al*., 2016). Host environment related changes, including seasonal variation (temperature, rainfall and humidity) affect the population dynamics of parasite and thus knowing how it affects can contribute to better understanding of possible control of diseases caused by the parasites and other factors related to health (Altizer *et al*., 2006; Burlet *et al*., 2011; Ahmad *et al*., 2014). Parasites also have a huge impact on hosts. Their life cycle and transmission pattern

study is useful for epidemiological purposes. They can also be indicators of host biology, ecosystem stress, food webs and biodiversity (Marcogliese, 2004).

One of the most important factors that influence seasonality is the temperature which has a direct impact on free-living developmental stages or the parasite within the hosts. Changes in the distribution of acanthocephalans with the change in seasons have been observed in many fish hosts (Gupta *et al*., 2012a; Sheema *et al*., 2015; Boping and Wenbin, 2007). This is also true for other helminth parasites like digenetic trematodes where the seasonal changes in the prevalence and abundance are related to cercarial emergence with an increase in water temperature (Imani-Baran *et al*., 2013).

Seasonal variation of helminth parasites, including parasites of public health importance in fishes has been studied by many authors from different parts of the world and their existence is influenced by many factors (Jorgensen *et al*., 2008; Carvalho and Luque, 2011; Mehrdana *et al*., 2014; Zhang *et al*., 2014; Uruku and Adikwu, 2017). In India, a seasonal study of different helminth parasite infection in fishes has been reported by several authors (Das and Goswami, 2014; Selvakumar *et al*., 2015; Wali *et al*., 2016; Fartade *et al*., 2017).

Manipur is one of the North-Eastern states of India with a rich biodiversity and where it also has a great variety of fishes. It has 167 species belonging to 84 genera, 31 families and 11 orders (Vishwanath, 2000). Now, it has increased up to 200 species, out of which 7 fishes are in IUCN red list (Vishwanath *et al*., 2007). However, very limited literatures are available on fish parasites from this region. Therefore, it is necessary to carry out a thorough investigation in this biogeographic region to evaluate the extent of helminthiases prevalent among some selected fishes.

The present study aimed to evaluate the seasonal variation in prevalence, abundance and mean intensity of the most frequently occurring helminth parasites among some selected fishes so as to understand and find out the possible preventive measure in this land lock state of Northeast India.

4.2 Materials and method

Collection of hosts and Locality

Fish hosts were collected in every alternate month for three years (Number of sampling = 18 from August 2014 - July 2017) as mentioned in chapter 1. The piscine hosts were collected **(alive)** from fishermen of various localities and rural markets. The localities surveyed includes Loktak, Moirang and Ningthoukong (Bishnupur district), Lamlong (Imphal East district), Lamphel, (Imphal West district), Kakching and Tentha (Thoubal district), and Kachai and Hungpung (Ukhrul district). The geographical co-ordinates of the mentioned districts are listed in the Table 4.1.

Table 4.1 Geographical co-ordinates of sampling sites

Locality	Latitude	Longtitude
Loktak	24.5593° N	93.8147° E
Moirang	24.4980° N	93.7765° E
Ningthoukong	24.5667° N	93.7587° E
Lamlong	24.7807° N	93.9674° E
Lamphel	24.8236° N	93.9114° E
Kakching	24.4969° N	93.9831° E

Tentha	24.5731° N	93.9725° E
Kachai	25.2354° N	94.2736° E
Hungpung	25.0954° N	94.3617° E

Seasonal studies

A total number of 2533 fishes i.e., 652 *Anabas testudineus*, 483 *Channa striata*, 400 *C. punctata*, 100 *C. gachua*, 530 *Clarias magur*, 73 *Trichogaster fasciata*, 105 *Monopterus cuchia*, 70 *Lepidocephalichthys guntea*, 70 *Heteropneustes fossilis* and 50 *Notopterus notopterus* were sampled during the period August 2014 - July 2017 from the aforementioned sites. The parasites collected from these fishes were counted, recorded and the seasonal study was carried out for the most frequently occurring parasites. The parasites viz., *Camallanus anabantis*, *Paraquimperia manipurensis*, *Neocamallanus singhi*, *Lytocestus attenuatus*, *Lytocestus indicus*, *Lytocestus filiformes*, *Lytocestus longicolis*, *Djombangia penetrans*, *Pallisentis ophiocephali* and *Pallisentis indicus* were found frequently in fishes like *C. striata*, *C. punctata*, *A. testudineus* and *C. magur*.

For statistical analysis, the division of three seasons in a year, i.e., Monsoon (June, July, August and September), Post-monsoon (October, November, December and January) and Pre-monsoon (February, March, April and May) was considered. Seasonal variation in prevalence, Abundance and Mean Intensity of the frequently occurring parasites were measured following Margolis *et al*. (1982):

$$\textbf{Prevalence} = \frac{\text{Number of hosts infected}}{\text{Number of hosts examined}} \times 100$$

Abundance = Number of parasites recovered

Number of hosts examined

Mean Intensity = Number of parasites recovered

Number of hosts infected

Percentage similarity

To calculate and compare the similarities between two communities, Sorenson's Index was used (Sorensen, 1948):

$S = 2C \times 100 / A+B$

Where,

S= percentage community similarity

A = number of species present in one community

B = number of species present in another community

C = number of species common in both

One-way Analysis of Variance (ANOVA) and Tukey's test

To know the significance of variations of prevalence, abundance and mean intensity with the seasons, ANOVA accompanied with Tukey's post hoc test was used. To determine the co-relation between the meteorological factors of different seasons and the prevalence, abundance and mean intensity of helminth infection and to ascertained the level of significance Pearson's correlation was carried out via www.vassarstats.net/tabs.html#z

4.3 Results

During the study period (August 2014 - July 2017), 2265 freshwater fishes (out of 2533 fishes) were examined for seasonal occurrence of helminth parasites and a total of 4735 helminths belonging to various taxa were found infecting them. The highest parasite infection was recorded from the taxa Lytocestidae followed by Quadrigyridae and Camallanidae (Table 4.2). The species composition of the helminth parasites from all the fishes examined are presented in Table 4.3.

The similarity of helminth parasite assemblage from one locality to another was compared using Sorensen's index. The highest percentage similarity was observed between Thoubal and Bishnupur whereas the lowest similarity was noticed between Ukhrul and Imphal East, Ukhrul and Imphal West (Table 4.4).

Table 4.2 Various group of helminth parasites recorded from piscine hosts of Manipur

Groups	Number of Specimens recovered	Genera
Camallanidae	1075	4
Quimperiidae	477	1
Anisakidae	25	1
Bothriocephalidae	15	1
Lytocestidae	1624	2
Plagiorchiidae	2	1
Clinostomidae	7	1
Quadrigyridae	1483	3

Echinorhynchidae	1	1
Gorgoderidae	10	1
Diplostomatidae	30	1
Total	4749	17

Table 4.3 Species composition of helminths in freshwater fishes of Manipur

Phylum: Platyhelminthes **Class: Cestoda**						
Taxa	**Host↓**	**Site**				
		1	**2**	**3**	**4**	**5**
Order: Caryophyllidea **Family: Lytocestidae**						
1. *Lytocestus attenuatus*	*Clarias magur*	+	+	+	+	-
2. *Lytocestus indicus*	*C. magur*	+	+	+	+	-
3. *Lytocestus filiformes*	*C. magur*	+	+	+	+	-
4. *Lytocestus longicollis*	*C. magur*	+	+	+	+	-
5. *Djombangia penetrans*	*C. magur*	+	+	+	+	-
Order: Pseudophyllidea **Family: Bothriocephalidae**						

6. *Senga lucknowensis*	*Channa punctata*	-	-	+	-	-
Phylum: Nemathelminthes **Class: Nematoda**						
Order: Spiruridea **Family: Camallanidae**						
7. *Camallanus anabantis*	*Anabas testudineus*	+	+	+	+	-
8**.** *Neocamallanus singhi*	*A. testudineus*	-	+	+	+	-
9. *Paracamallanus ophiocephali*	*A. testudineus*	+	+	+	+	-
10. *Procamallanus sp.*	*A. testudineus*	+	+	+	+	-
Anisakidae						
11. *Anisakis* sp.	*Channa gachua*	_	_	+	+	+
Order: Ascaridoidea **Family: Quimperiidae**						
12. *Paraquimperia manipurensis*	*A. testudineus*	+	+	+	+	-
Phylum: Acanthocephalans **Class: Eoacanthocephala**						
Order: Gyracanthocephala **Family: Quadrigyridae**						
13. *Pallisentis ophiocephali*	*C. striata, C. punctata*	+	+	+	+	-

1:Imphal East, 2:Imphal West, 3:Thoubal, 4:Bishnupur, 5:Ukhrul.

Table continuation

14. *Pallisentis indicus*	*C. striata, C. punctata*	+	+	+	+	-

15. *Pallisentis sp.*	*C. striata, C. punctata*	-	+	-	-	-
Order: Neoechinorhynchida **Family: Neoechinorhynchidae**						
16. *Neoechinorhynchus sp.*	*C. striata*	-	-	-	+	-
Order: Echinorhynchida **Family: Echinorhynchidae**						
17. *Echinorhynchus sp.*	*C. striata*	-	-	-	+	-
Phylum: Platyhelminthes **Class: Trematoda**						
Order: Clinostomoidea **Family: Clinostomidae**						
18. *Clinostomum sp*.	*Trichogaster fasciata*	-	+	-	-	-
Order: Plagiorchioidea **Family: Plagiorchiidae**						
19. *Astiotrema reniferum*	*C. magur*	-	+	-	-	-
Order: Plagiorchioidea **Family: Gorgoderidae**						
20. *Phyllodistomum sp.*	*C. gachua*	-	-	-	+	-
Order: Diplostomoidea **Family: Diplostomatidae**						
21. *Posthodiplostomum sp.*	*C. gachua, C. punctata*	-	+	+	+	-
Total		**11**	**16**	**15**	**17**	**01**

1:Imphal East, 2:Imphal West, 3:Thoubal, 4:Bishnupur, 5:Ukhrul.

Table 4.4 Percentage similarity of helminth assemblages in different collection sites of Manipur

Sites	1	2	3	4	5
1	ID	84.6	84.6	78.6	0.0
2		ID	86.7	81.3	0.0
3			ID	87.5	12.5
4				ID	11.1
5					ID

[Note: ID= Identical]

1 - Imphal East, 2 - Imphal West, 3 - Thoubal, 4 - Bishnupur, 5 - Ukhrul.

Statistical analysis

The meteorological factors like temperature, rainfall and humidity of all the three seasons (Monsoon, Post-monsoon and Pre-monsoon) were considered to study their effect on the seasonal changes in the prevalence, abundance and mean intensity of all the frequently occurring parasites (Table 4.5) and their Co-relation co-efficients were also calculated to check the significance (Table 4.7, Table 4.9)

Table 4.5 Meteorological factors (average temperature, average rainfall and average relative humidity) in the state of Manipur during the study period (August 2014 - July 2017)

Year	Season (°C)	Temperature (in mm)	Rainfall (%)	Humidity
2014-15	Monsoon	24.7	240.31	82.90
	Post-monsoon	18.6	29.42	73.70
	Pre-monsoon	20.2	130.72	68.60
2015-16	Monsoon	26.9	297.06	83.00
	Post-monsoon	20.3	63.41	71.03
	Pre-monsoon	21.7	82.53	74.45
2016-17	Monsoon	27.9	328.0	84.10
	Post-monsoon	19.2	83.50	72.07
	Pre-monsoon	21.4	201.0	75.60

The climatic variables were taken from GIS enabled meteorological database of National Aeronautics and Space Administration (NASA) surface meteorology and solar energy: RET screen data (https://eosweb.larc.nasa.gov/sse/RETScreen/) Indian council of agriculture research (ICAR) Lamphelpat, Manipur.

Host wise studies

During the three years (August 2014 - July 2017) of study, a total number of 652 *A. testudineus*, 530 *C. magur* , 483 *C. striata* and 400 *C. punctata* were dissected and seasonal variations in prevalence, abundance and mean intensity of helminth parasites were studied. The prevalence values were above 39.75% in all seasons with distinct seasonal patterns of occurrence.

Anabas testudineus

The prevalence was revealed to be highest during pre-monsoon, followed by monsoon and post-monsoon, whereas the abundance and mean intensity was observed highest in monsoon. The abundance was lowest in post-monsoon and mean intensity were lower in pre-monsoon (Table 4.6; Fig. 4.1).

The meteorological factors, i.e., temperature, rainfall and humidity showed positive co-relation with prevalence, abundance and mean intensity (Table 4.7).

Clarias magur

The prevalence of the taxa Lytocestidae in *C. magur* peaked in monsoon, followed by post monsoon and dropped significantly in pre-monsoon whereas the abundance and mean intensity was observed highest in post-monsoon (Table 4.6; Fig. 4.2).

Co-relation co-efficient results showed that the factors, temperature, rainfall and humidity have a significant negative co-relation to mean intensity and positive co-relation with prevalence whereas rainfall has negative co-relation with abundance and temperature and humidity has positive relation with abundance (Table 4.7).

Channa striata

The prevalence was seen highest during pre-monsoon followed by monsoon and post- monsoon whereas abundance and mean intensity were seen highest during monsoon. The abundance was observed to be lowest in post-monsoon whereas mean intensity was lowest in pre-monsoon (Table 4.6; Fig. 4.3).

Temperature, rainfall and humidity have a significant positive co-relation with abundance and mean intensity, whereas prevalence has negative co-relation with humidity and positive co-relation to temperature and rainfall (Table 4.7).

Channa punctata

Sseasonal variations in prevalence, abundance and mean intensity of helminth parasites of *Channa punctata* were studied. The prevalence was observed highest in pre-monsoon followed by monsoon and post-monsoon. Abundance and mean intensity highest in monsoon (Table 4.6; Fig. 4.4).

Temperature, rainfall and humidity showed positive co-relation with abundance and mean intensity, whereas temperature and humidity showed negative co-relation with prevalence (Table 4.7).

Table 4.6 Overall prevalence, abundance and mean intensity of different parasites among different fishes collected in different seasons during the study period (August 2014 - July 2017)

FISH HOST	SEASONS	PREVALENCE (%)	ABUNDANCE	MEAN INTENSITY
A. testudineus	Monsoon	68.31±1.69	2.59±0.26	3.79±0.33
	Post-monsoon	59.87±0.68	2.15±0.32	3.58±0.50
	Pre-Monsoon	74.95±1.53	2.54±0.24	3.38±0.30
C. magur	Monsoon	76.07±1.48	1.99±0.04	2.56±0.03
	Post-monsoon	71.42±0.56	2.20±0.08	3.09±0.10
	Pre-Monsoon	39.75±1.04	1.13±0.01	2.86±0.06
C. striata	Monsoon	68.24±8.30	1.52±0.14	2.26±0.15
	Post-monsoon	63.33±3.33	0.85±0.07	1.36±0.16
	Pre-Monsoon	83.56±1.93	1.01±0.02	1.21±0.05
C. punctata	Monsoon	64.17±3.01	1.41±0.07	2.20±0.01
	Post-monsoon	55.71±0.28	1.05±0.25	1.88±0.45

	Pre-Monsoon	81.83±3.67	1.08±0.03	1.33±0.03

Table 4.7 Co-relation co-efficient (r) of prevalence, abundance and mean intensity of helminth infections recorded in different freshwater fishes of Manipur with meteorological parameters '+' indicates significant positive correlation and '-' indicates significant negative correlation (*p≤ 0.05, **p≤ 0.01 and ***p≤ 0.001)

		Temperature	Rainfall	Humidity
Anabas testudineus	Prevalence	+0.32	+0.46	+0.12
	Abundance	+0.46	+0.55	+0.41
	Mean intensity	+0.32	+0.35	+0.37
Clarias magur	Prevalence	+0.37	+0.27	+0.51
	Abundance	+0.08	–0.03	+0.26
	Mean intensity	–0.83**	–0.93***	–0.72*
Channa striata	Prevalence	+0.11	+0.18	–0.19
	Abundance	+0.93***	+0.90***	+0.84*
	Mean intensity	+0.76**	+0.72*	+0.86**
Channa punctata	Prevalence	–0.001	+0.02	–0.12
	Abundance	+0.43	+0.40	+0.64
	Mean intensity	+0.33	+0.28	+0.57

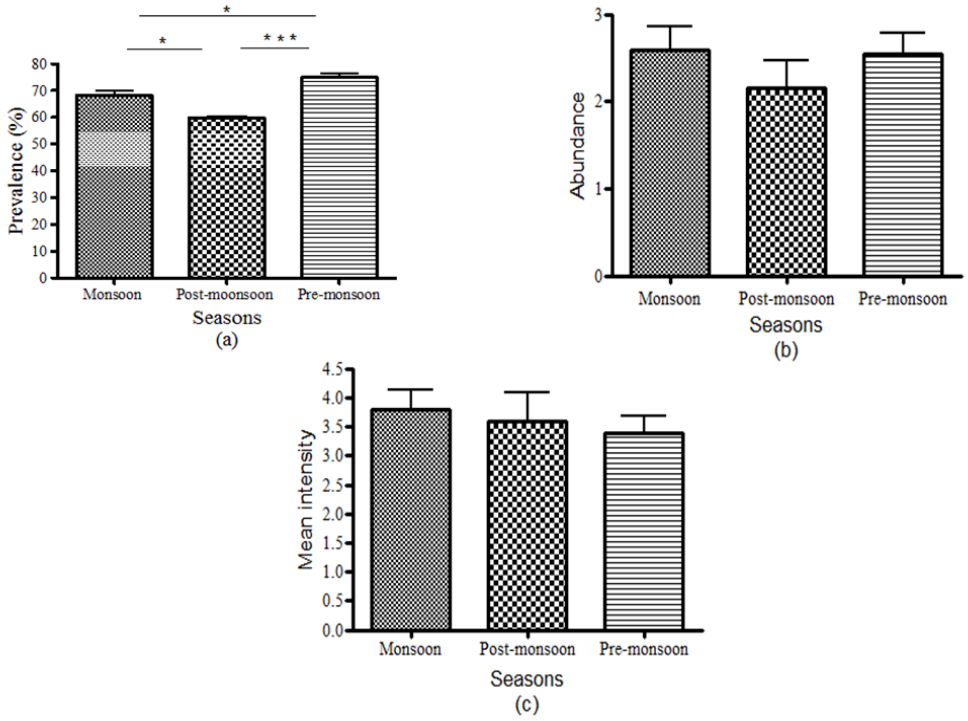

Fig. 4.1 Seasonal fluctuations of different helminth parasites in *Anabas testudineus*: (a) Prevalence, (b) Abundance and (c) Mean intensity. Values are expressed as Mean± SEM (N= 6). * p≤ 0.05, ** p≤ 0.01, *** p≤ 0.001. One-way ANOVA, Tukey Test

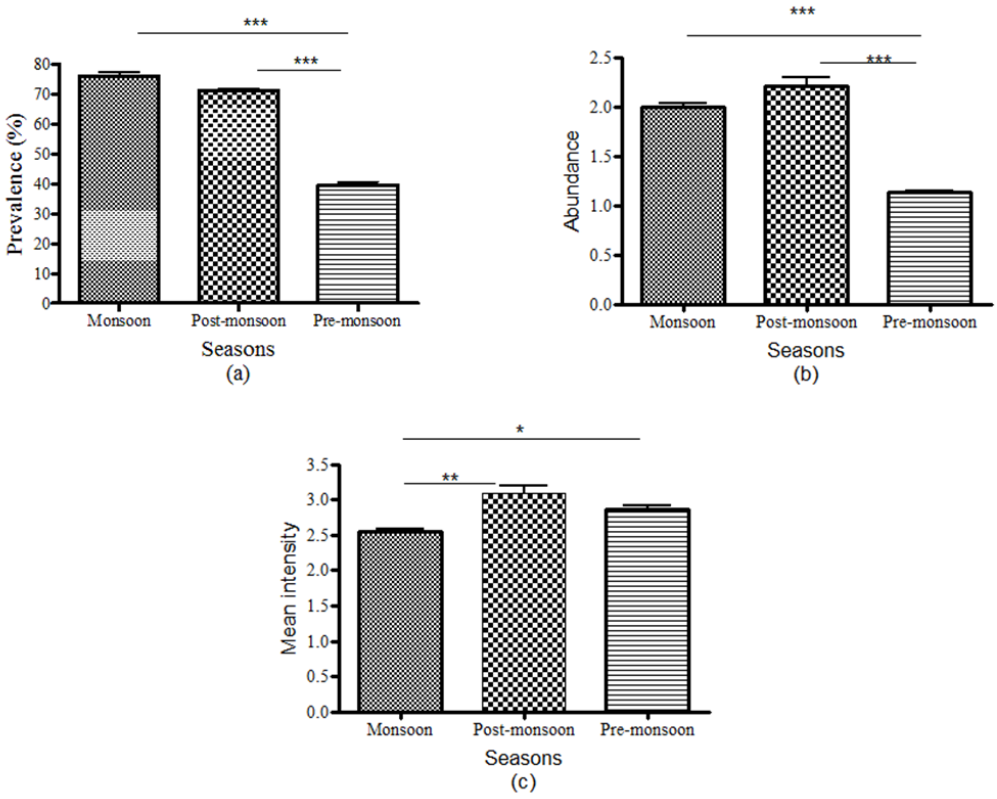

Fig. 4.2 Seasonal fluctuations of different helminth parasites in *Clarias magur*: (a) Prevalence, (b) Abundance and (c) Mean intensity. Values are expressed as Mean± SEM (N= 6). * p≤ 0.05, ** p≤ 0.01, *** p≤ 0.001 One-way ANOVA, Tukey Test

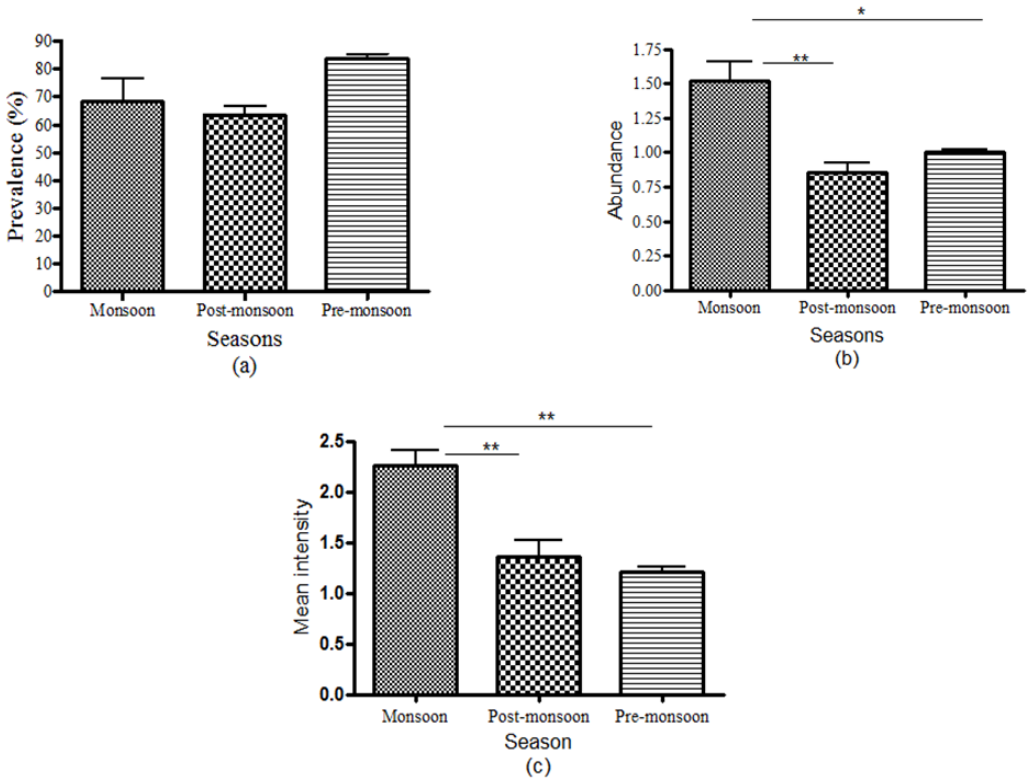

Fig. 4.3 Seasonal fluctuations of different helminth parasites in *Channa striata*: (a) Prevalence, (b) Abundance and (c) Mean intensity. Values are expressed as Mean± SEM (N= 6). * p≤ 0.05, ** p≤ 0.01. One-way ANOVA, Tukey Test

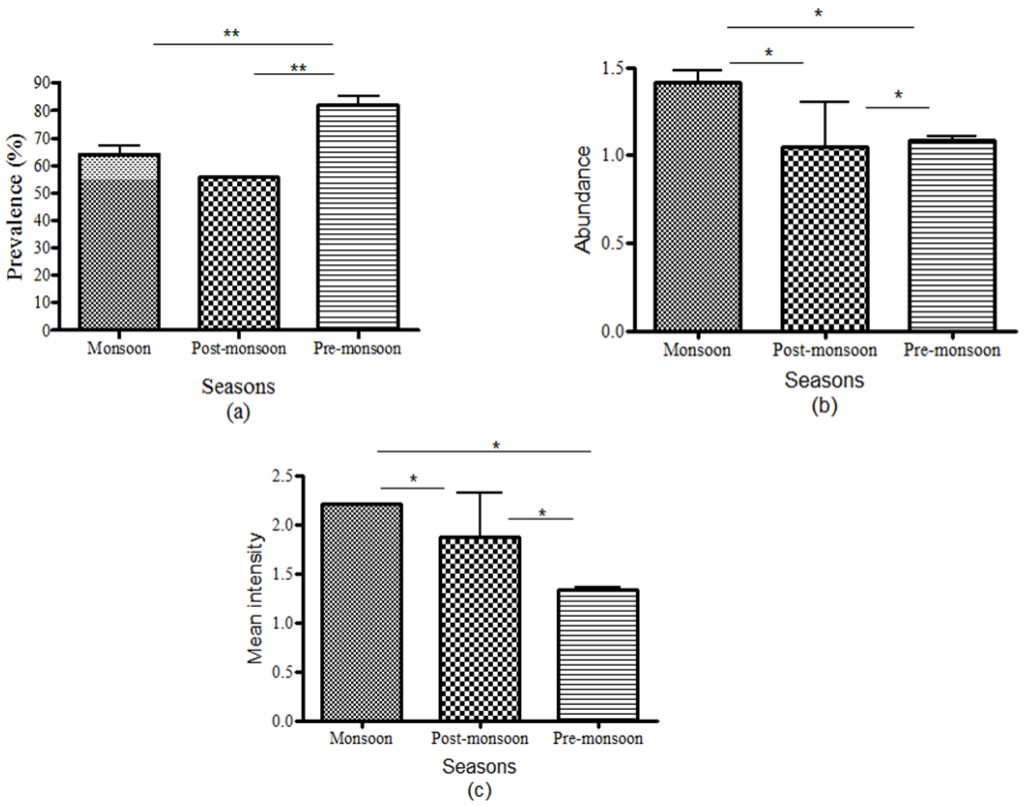

Fig. 4.4 Seasonal fluctuations of different helminth parasites in *Channa punctata*: (a) Prevalence, (b) Abundance and (c) Mean intensity Values are expressed as Mean± SEM (N= 6). *p≤ 0.05, ** p≤ 0.01. One-way ANOVA, Tukey Test

Parasite wise studies

A total of 4735 helminths were collected from different fish hosts and seasonal variations in prevalence, abundance and mean intensity of the most frequently occurring parasites were studied. Rate of prevalence due to different parasites ranges from 9.93 % to 81% (Table 4.8)

Camallanus anabantis

Prevalence peaked in pre-monsoon followed by monsoon and post-monsoon. Abundance and mean intensity was observed to be highest in monsoon whereas lowest in post-monsoon and pre-monsoon, respectively (Table 4.8; Fig. 4.5).

Temperature and humidity showed negative co-relation with prevalence whereas rainfall showed positive co-relation. All the three climatic factors showed positive co-relation with abundance and mean intensity and the significance is seen with rainfall and humidity (Table 4.9).

Paraquimperia manipurensis

Prevalence value was highest in pre-monsoon, followed by post-monsoon and monsoon. Abundance was highest in pre-monsoon and lowest in post-monsoon. Mean intensity peaked in monsoon and reduced in pre-monsoon and dropped lowest in post-monsoon (Table 4.8; Fig. 4.6)

A significant negative co-relation was observed between prevalence and temperature, and prevalence and humidity. Though abundance showed positive co-relation with all the climatic factors, it is significant only for temperature. Mean intensity of the parasite showed significant co-relation for all the climatic factors (Table 4.9).

Neocamallanus singhi

Prevalence of the parasite during monsoon and post-monsoon did not show much difference however it increased during pre-monsoon. The abundance and mean intensity was also observed highest during pre-monsoon and lowest in post-monsoon and monsoon, respectively (Table 4.8; Fig. 4.7)

All the three factors i.e., temperature, rainfall and humidity showed negative co-relation with abundance and mean intensity. The value of prevalence did not show any co-relation with temperature however, it showed positive co-relation with rainfall and negative co-relation with humidity (Table. 4.9).

Lytocestus attenuatus

The prevalence and abundance were highest during monsoon followed by post-monsoon and dropped considerably during pre-monsoon whereas the mean intensity was highest during post-monsoon followed by pre-monsoon and monsoon (Table 4.8; Fig. 4.8).

The prevalence and abundance showed positive co-relation with temperture, rainfall and humidity but were not statistically significant except for humidity with prevalence. A significant negative co-relation exist between mean intensity and rainfall (Table 4.9).

Lytocestus indicus

The seasonal variation pattern of prevalence and mean intensity of *L. indicus* is similar to *L. attenuatus* where the highest value of prevalence was during monsoon followed by post-monsoon and pre-monsoon and mean intensity was highest during post-monsoon and lowest in monsoon (Table. 4.8; Fig. 4.9).

Temperature, rainfall and humidity have positive co-relation to prevalence and abundance but statistically significant co-relation was observed only for prevalence to temperature and humidity. Temperature has a significant negative co-relation to mean intensity (Table. 4.9).

Lytocestus filiformes

Prevalence of the parasite was highest during monsoon followed by post-monsoon and pre-monsoon. However, abundance and mean intensity were highest in post-monsoon (Table. 4.8; Fig. 4.10).

Only temperature and humidity showed significant positive co-relation with prevalence. (Table 4.9). All the parameters showed negative co-relation with mean intensity. However, abundance of the parasite showed positive co-relation with humidity, though it is not statistically significant (Table 4.9).

Lytocestus longicollis

The prevalence and abundance of *L. longicollis* were observed highest during monsoon and reduces in post-monsoon and pre-monsoon whereas the mean intensity was highest in pre-monsoon followed by post-monsoon and monsoon (Table 4.8; Fig. 4.11).

All the three factors i.e., temperature rainfall and humidity showed positive co-relation to prevalence and abundance but significant co-relation was observed only for humidity. Mean intensity showed negative co-relation to all the factors but not statistically significant (Table 4.9).

Djombangia penetrans

Prevalence and abundance of *D. penetrans* were highest during monsoon and lowest in pre-monsoon whereas mean intensity was highest in post-monsoon followed by pre-monsoon and monsoon (Table 4.8; Fig. 4.12).

A significant positive co-relation were observed between prevalence and temperature, and prevalence and humidity. However, mean intensity showed negative co-relation for all the factors (Table 4.9).

Pallisentis ophiocephali

The prevalence peaked in pre-monsoon, reduces during monsoon and dropped significantly during post-monsoon whereas abundance and mean intensity were higher during monsoon and lowest during post-monsoon and pre-monsoon (Table 4.8; Fig. 4.13).The climatic variables i.e., temperature, rainfall and humidity showed a significant positive co-relation with abundance and mean intensity (Table 4.9).

Pallisentis indicus

Prevalence and abundance of *P. indicus* were highest during pre-monsoon whereas highest mean intensity of the parasite was recorded during post-monsoon (Table 4.8; Fig. 4.14).

Temperature, rainfall and humidity showed significant negative co-relation with mean intensity, abundance of the parasite also showed negative co-relation with all the factors, but not statistically significant. Prevalence of *P. indicus* revealed positive co-relation with temperature and rainfall, but not statistically significant (Table. 4.9).

Table 4.8 Species-wise prevalence, abundance and mean intensity of different helminth parasites collected in different seasons during the study period

Parasites	Seasons	Prevalence (%)	Abundance	Mean intensity
Camallanus anabantis	Monsoon	57.36±1.32	1.25±0.22	2.16±0.32
	Post-monsoon	53.81±1.85	0.91±0.14	1.71±0.19
	Pre-monsoon	70.13±1.16	1.00±0.15	1.43±0.18
Paraquimperia manipurensis	Monsoon	36.40±1.43	0.80±0.07	2.24±0.28
	Post-monsoon	39.52±1.59	0.58±0.03	1.51±0.14
	Pre-monsoon	43.78±1.52	0.81±0.08	1.88±0.26
Neocamallanus singhi	Monsoon	21.94±1.08	0.49±0.03	2.26±0.19
	Post-monsoon	18.92±0.30	0.47±0.01	2.51±0.04
	Pre-monsoon	28.70±0.74	0.70±0.02	2.54±0.16
Lytocestus attenuatus	Monsoon	28.85±1.07	0.56±0.18	1.45±0.19
	Post-monsoon	20.42±1.23	0.46±0.03	2.27±0.01
	Pre-monsoon	9.93±0.78	0.17±0.04	1.80±0.26
Lytocestus indicus	Monsoon	45.26±0.75	0.77±0.01	1.72±0.04
	Post-monsoon	37.95±0.46	0.82±0.02	2.16±0.04
	Pre-monsoon	30.16±0.74	0.54±0.01	1.79±0.08
Lytocestus filiformes	Monsoon	32.52± 1.34	0.45±0.02	1.41±0.12
	Post-monsoon	24.49±1.44	0.58±0.02	2.43±0.19
	Pre-monsoon	18.60±1.45	0.26±0.01	1.42±0.05
Lytocestus longicollis	Monsoon	31.80±4.07	0.41±0.04	1.29±0.03
	Post-monsoon	21.22±0.75	0.31±0.02	1.52±0.09
	Pre-monsoon	14.86±1.10	0.30±0.04	2.14±0.41

Djombangia penetrans	Monsoon	25.07±1.00	0.49±0.06	2.00±0.34
	Post-monsoon	18.35±0.57	0.42±0.02	2.30±0.07
	Pre-monsoon	10.33±0.46	0.21±0.02	2.11±0.14
Pallisentis ophiocephali	Monsoon	72.54±1.33	1.49±0.02	2.06±0.01
	Post-monsoon	59.68±1.73	0.79±0.07	1.34±0.15
	Pre-monsoon	81.06±1.70	1.04±0.01	1.27±0.01
Pallisentis indicus	Monsoon	30.44±0.95	0.42±0.02	1.41±0.07
	Post-monsoon	25.53±1.13	0.48±0.01	1.92±0.06
	Pre-monsoon	40.06±0.61	0.73±0.02	1.84±0.04

Table 4.9 Correlation coefficient (r) of prevalence, abundance and mean intensity of different helminth infections with meteorological parameters '+' indicates significant positive correlation and '-' indicates significant negative correlation (*p≤ 0.05, **p≤ 0.01 and ***p≤ 0.001)

		Temperature	Rainfall	Humidity
Camallanus anabantis	Prevalence	–0.05	+0.10	–0.20
	Abundance	+0.60	+0.69*	+0.59
	Mean intensity	+0.63	+0.65*	+0.67*
Paraquimperia manipurensis	Prevalence	–0.64*	–0.58	–0.69*
	Abundance	+0.65*	+0.63	+0.53
	Mean intensity	+0.80**	+0.76*	+0.70*
Neocamallanus singhi	Prevalence	+0.0	+0.06	–0.16
	Abundance	–0.11	–0.03	–0.26

	Mean intensity	–0.2	–0.18	–0.27
Lytocestus attenuatus	Prevalence	+0.61	+0.54	+0.77**
	Abundance	+0.40	+0.32	+0.49
	Mean intensity	–0.58	–0.67*	–0.46
Lytocestus indicus	Prevalence	+0.65*	+0.53	+0.75**
	Abundance	+0.10	+0.03	+0.29
	Mean intensity	–0.69*	–0.62	–0.53
Lytocestus filiformes	Prevalence	+0.65*	+0.51	+0.78**
	Abundance	–0.10	–0.20	+0.07
	Mean intensity	–0.57	–0.57	–0.46
Lytocestus longicollis	Prevalence	+0.58	+0.52	+0.70*
	Abundance	+0.47	+0.38	+0.73*
	Mean intensity	–0.29	–0.30	–0.23
Djombangia penetrans	Prevalence	+0.69*	+0.57	+0.75**
	Abundance	+0.38	+0.30	+0.57
	Mean intensity	–0.45	–0.38	–0.19
Pallisentis ophiocephali	Prevalence	+0.33	+0.35	+0.13
	Abundance	+0.91***	+0.91***	+0.85**
	Mean intensity	+0.84**	+0.83**	+0.88***
Pallisentis indicus	Prevalence	+0.09	+0.17	–0.11
	Abundance	–0.35	–0.26	–0.50
	Mean intensity	–0.85**	–0.81**	–0.79**

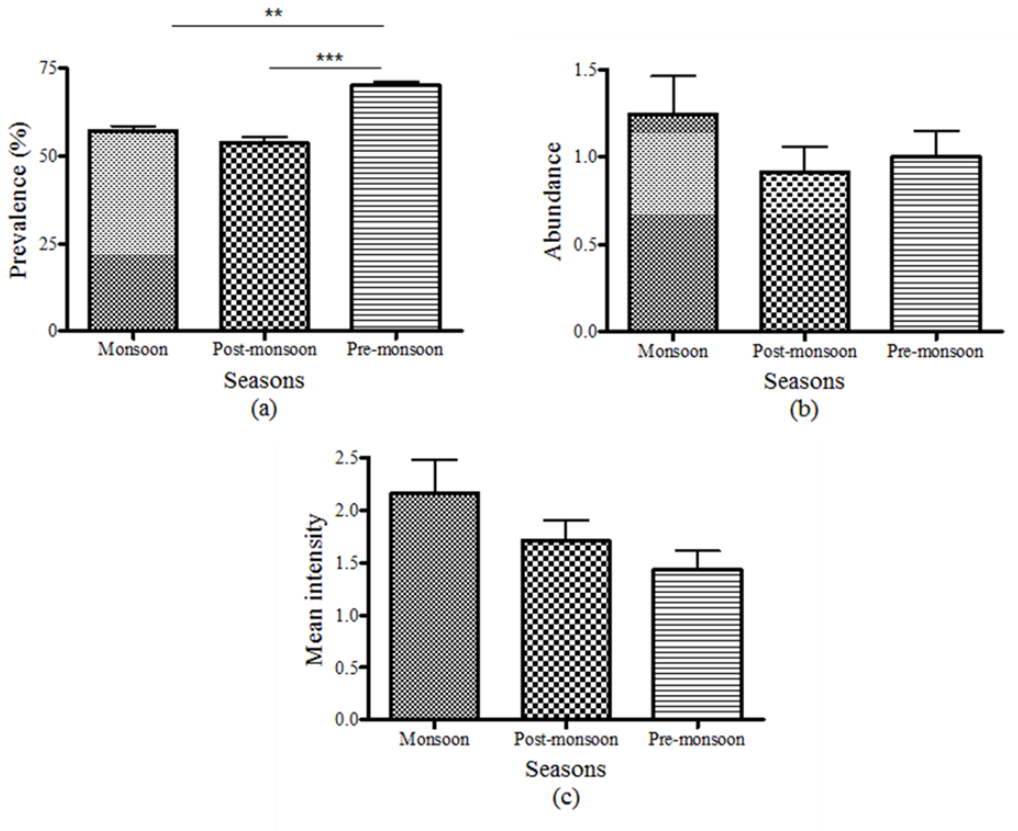

Fig. 4.5 Seasonal fluctuations of *Camallanus anabantis* in different fishes of Manipur: (a) Prevalence, (b) Abundance and (c) Mean Intensity. Values are expressed as Mean± SEM (N= 6). ** p≤ 0.01, *** p≤ 0.001. One-way ANOVA, Tukey's Test

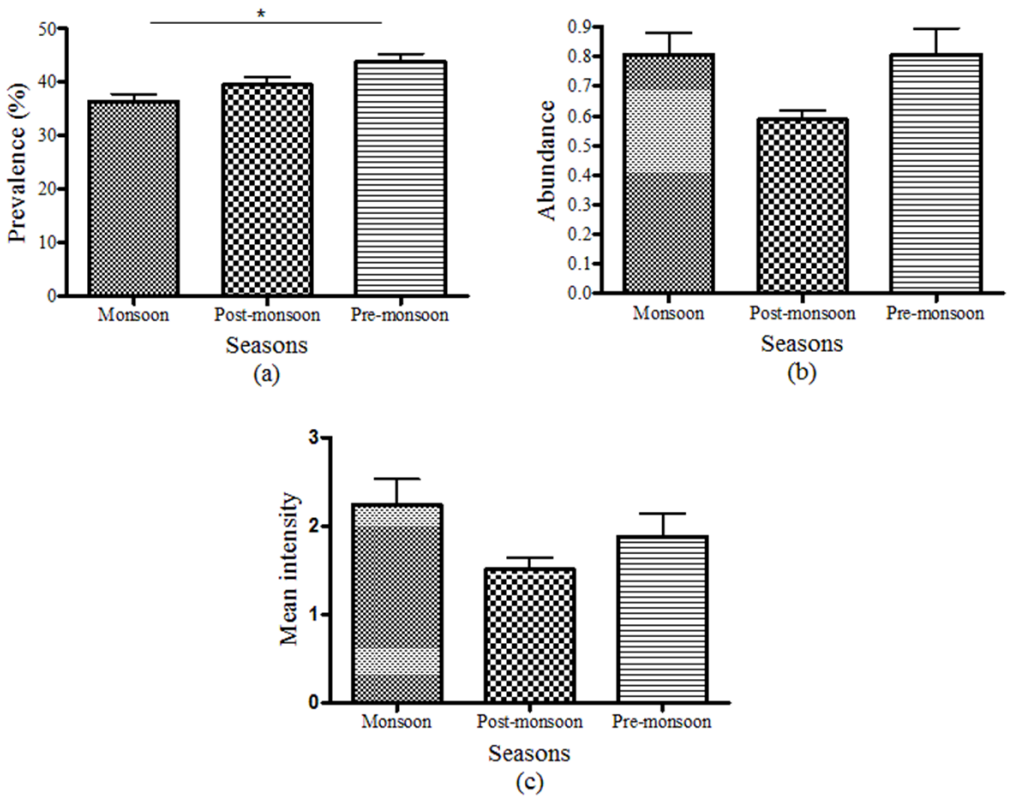

Fig. 4.6 Seasonal fluctuations of *Paraquimperia manipurensis* in different fishes of Manipur: (a) Prevalence, (b) Abundance and (c) Mean Intensity. Values are expressed as Mean± SEM (N= 6). * p≤ 0.05. One-way ANOVA, Tukey's Test

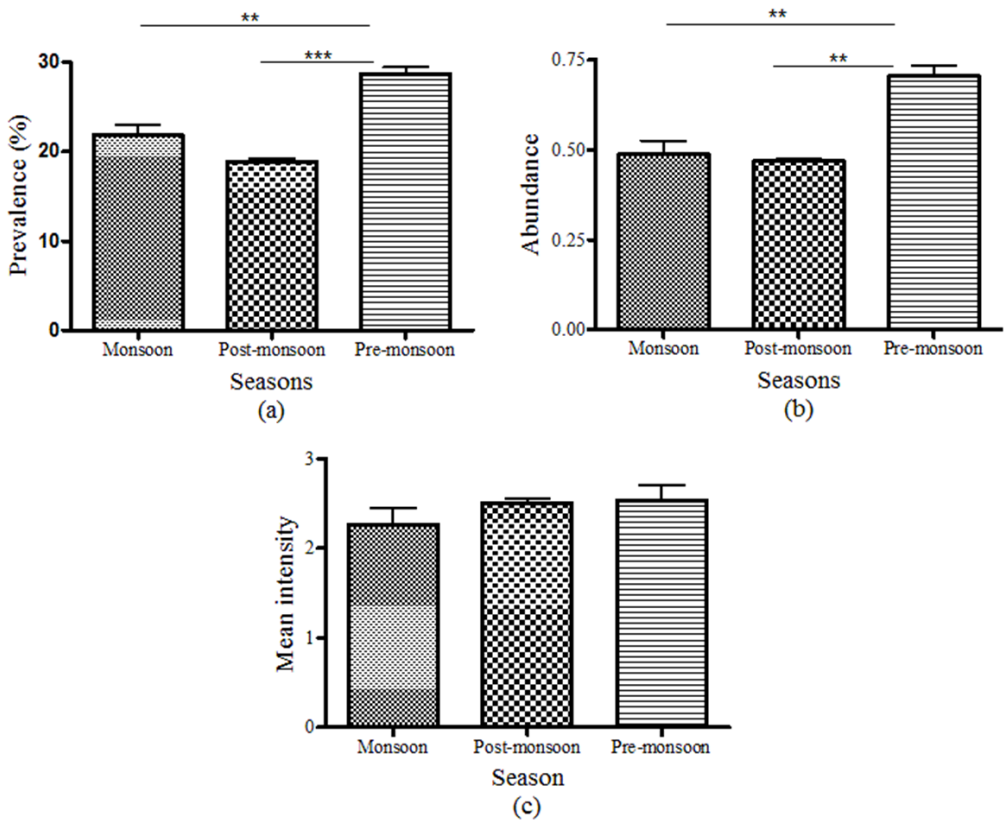

Fig. 4.7 Seasonal fluctuations of *Neocamallanus singhi* in different fishes of Manipur: (a) Prevalence, (b) Abundance and (c) Mean Intensity. Values are expressed as Mean± SEM (N= 6). ** p≤ 0.01, *** p≤ 0.001. One-way ANOVA, Tukey's Test

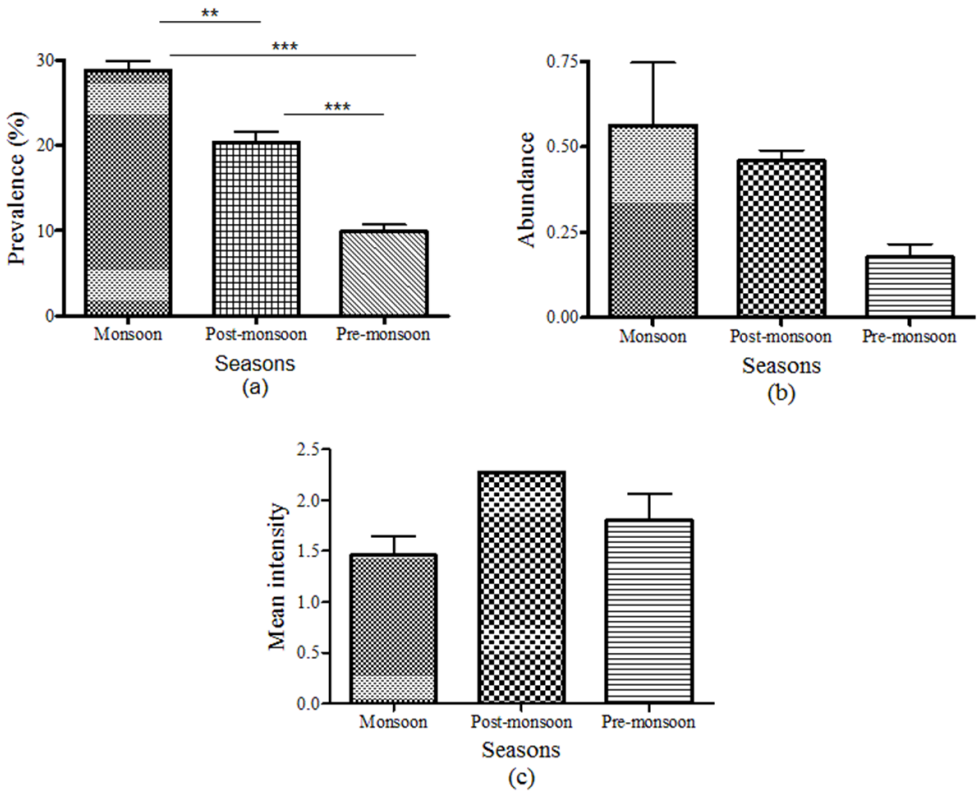

Fig. 4.8 Seasonal fluctuations of *Lytocestus attenuatus* in different fishes of Manipur: (a) Prevalence, (b) Abundance and (c) Mean Intensity. Values are expressed as Mean± SEM (N= 6). ** p≤ 0.01, *** p≤ 0.001. One-way ANOVA, Tukey's Test

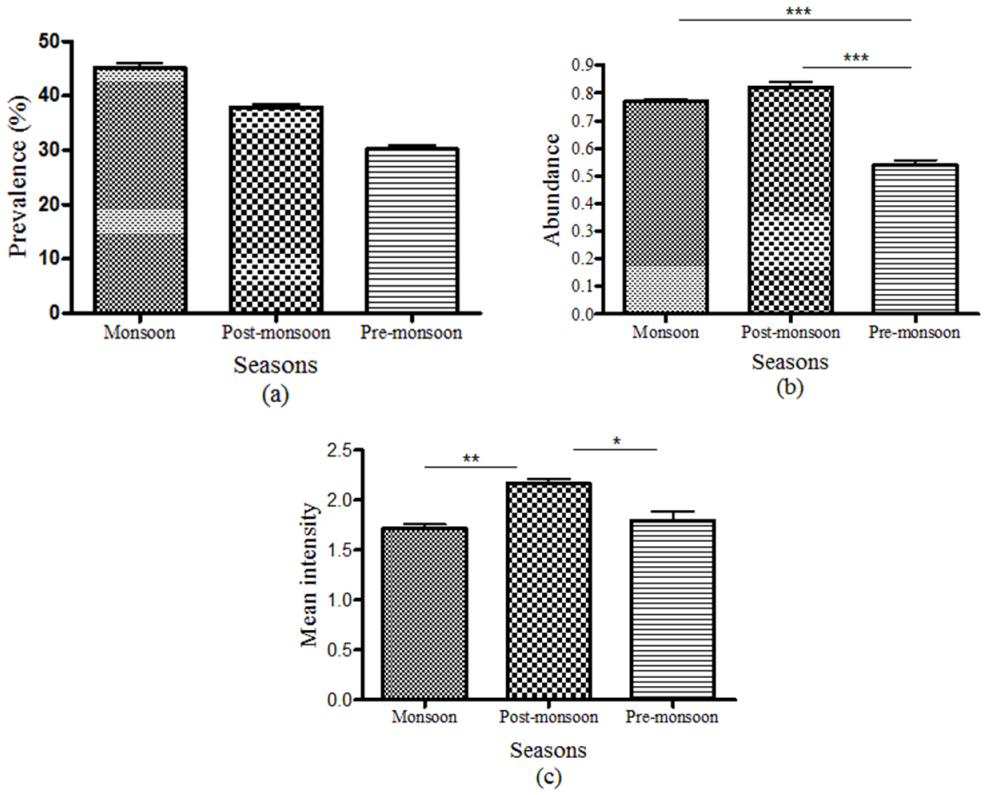

Fig. 4.9 Seasonal fluctuations of *Lytocestus indicus* in different fishes of Manipur: (a) Prevalence, (b) Abundance and (c) Mean Intensity. Values are expressed as Mean± SEM (N= 6). *p≤ 0.05, ** p≤ 0.01, *** p≤ 0.001. One-way ANOVA, Tukey's Test

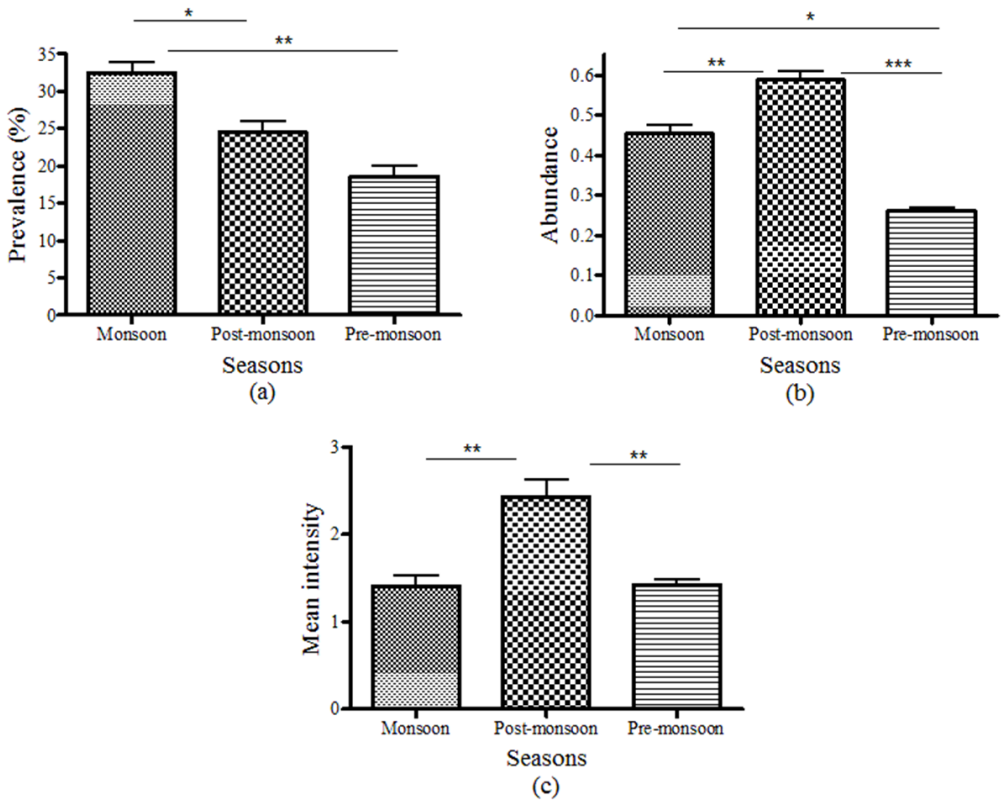

Fig. 4.10 Seasonal fluctuations of *Lytocestus filiformes* in different fishes of Manipur: (a) Prevalence, (b) Abundance and (c) Mean Intensity. Values are expressed as Mean± SEM (N= 6). *p≤ 0.05, ** p≤ 0.01, *** p≤ 0.001. One-way ANOVA, Tukey's Test

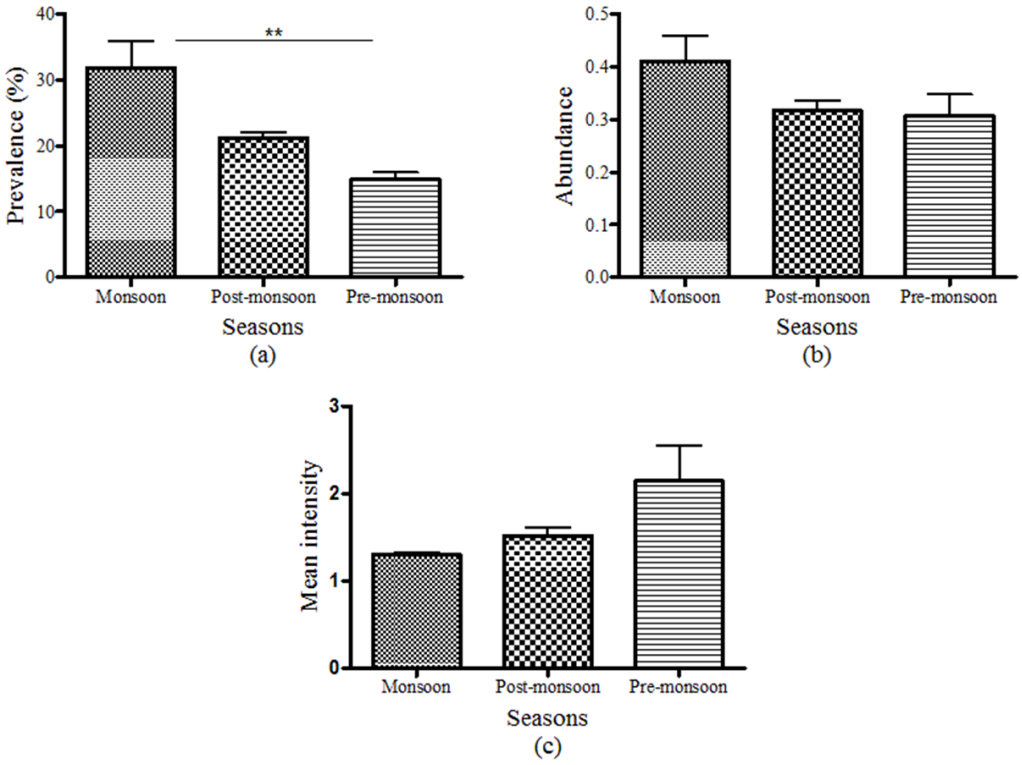

Fig. 4.11 Seasonal fluctuations of *Lytocestus longicollis* in different fishes of Manipur: (a) Prevalence, (b) Abundance and (c) Mean Intensity. Values are expressed as Mean± SEM (N= 6). ** p≤ 0.01. One-way ANOVA, Tukey's Test

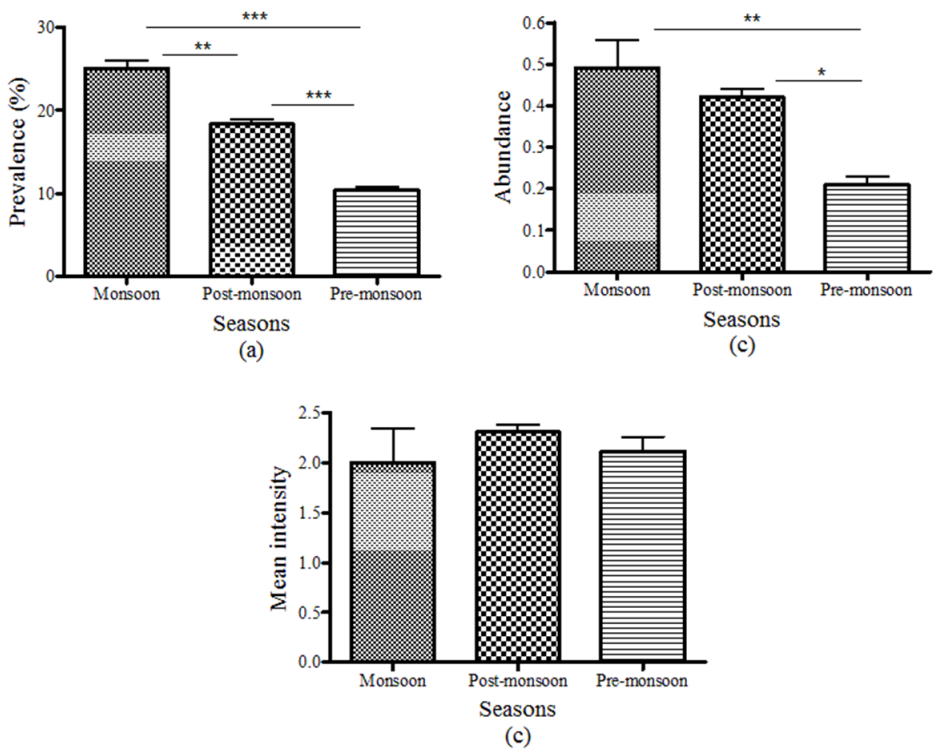

Fig. 4.12 Seasonal fluctuations of *Djombangia penetrans* in different fishes of Manipur: (a) Prevalence, (b) Abundance and (c) Mean Intensity. Values are expressed as Mean± SEM (N= 6). *p≤ 0.05, ** p≤ 0.01, *** p≤ 0.001. One-way ANOVA, Tukey's Test

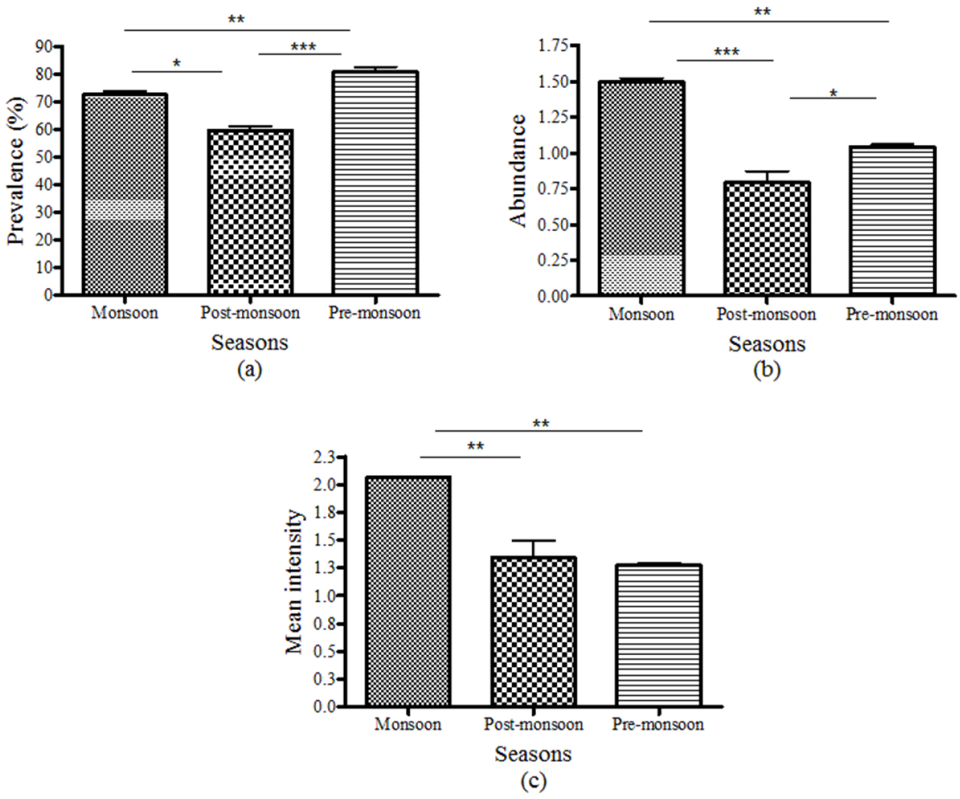

Fig 4.13. Seasonal fluctuations of *Pallisentis ophiocephali* in different fishes of Manipur: (a) Prevalence, (b) Abundance and (c) Mean Intensity. Values are expressed as Mean± SEM (N= 6). *p≤ 0.05, ** p≤ 0.01, *** p≤ 0.001. One-way ANOVA, Tukey's Test

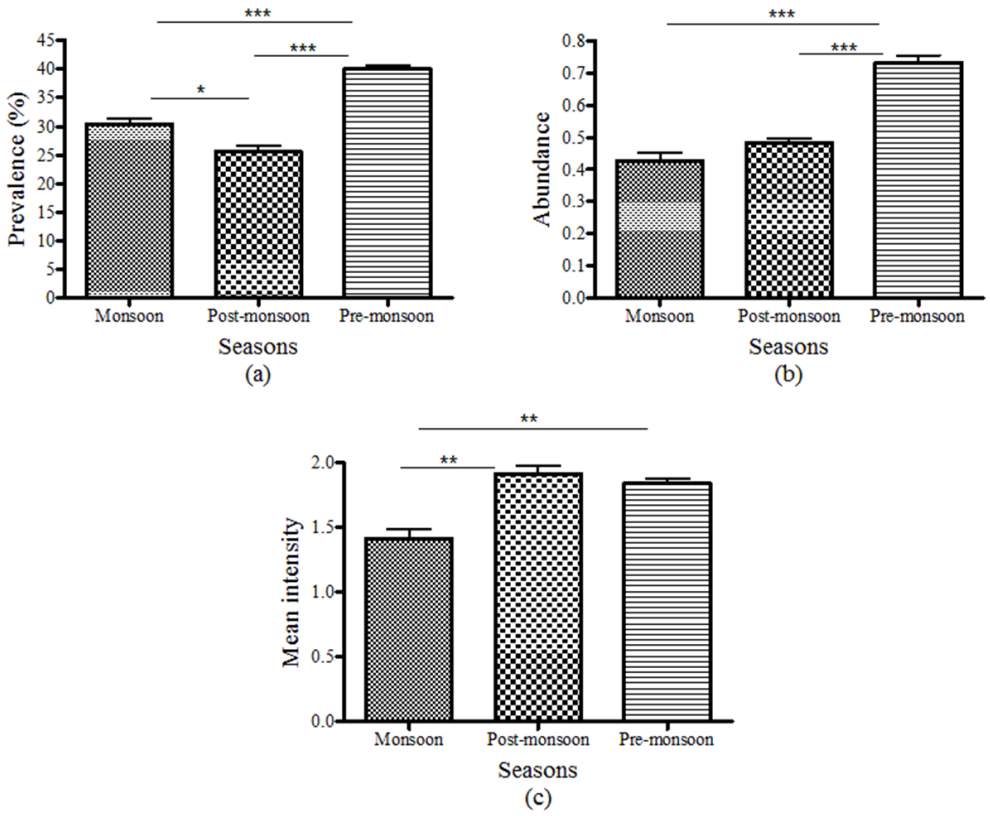

Fig. 4.14 Seasonal fluctuations of *Pallisentis indicus* in different fishes of Manipur: (a) Prevalence, (b) Abundance and (c) Mean Intensity. Values are expressed as Mean± SEM (N= 6). *p≤ 0.05, ** p≤ 0.01, *** p≤ 0.001. One-way ANOVA, Tukey's Test

4.4 Discussion

The parasite composition of the *Clarias magur* included five species of Lytocestidae (cestodes), one species of Plagiorchiidae (trematode) but no nematodes and acanthocephalans. In Perciformes fish, four species of the taxa Camallanidae (nematode), one Quimperiidae (nematode) and one Clinostomidae were recovered however, no cestodes and acanthocephalans were recorded. But, one cestode of the family Bothriocephalidae, one nematode species, four species of acanthocephalans and two species of trematode were collected from Channid fishes, thus contributing the highest number of parasite infestation in the present study.

The result of the percentage similarity of helminth assemblage in all the collection sites except site no. 5 i.e., Ukhrul district ranges from 7.86%-87.5%. The similarity percentage of Ukrul with other collection sites was very low (0-12%). The disparity in the similarity index may be due to the differences in the geographical topography where collection site 1-4 (Imphal East, Imphal West, Thoubal, Bishnupur) are valleys and relatively warmer than hilly Ukhrul region. The study conducted by Macnab and Barber (2012) and Sheath *et al*. (2016) suggested a similar type of results where fish parasites grow faster in warmer temperature than colder water temperature and also showed that there is a strong effect of temperature on parasite prevalence. Moreover, the work of Hu *et al*. (2011) and Soininen *et al*. (2007) demonstrated that the species richness and similarity relationship with the altitudinal gradients mostly follow a decreasing order.

The host-wise observations showed that prevalence, abundance and mean intensity observed highest in pre-monsoon in case of *A. testudineus, C. striata* and *C. punctata.* This could be due to the fact that the host's immune systems are weakened and nutrition

required for reproduction become poorer which could affect their defense system during pre-monsoon i.e., end of dry season and onset of spring (Altizer *et al*., 2006); whereas, in *C. magur*, prevalence was highest in monsoon and abundance and mean intensity was highest in post-monsoon. High rate of *Lytocestus* infection in *C. magur* during post monsoon i.e., the driest season of the year as observed in the present study may be related to hydrological cycle of the region where precipitation is negligible and thus the volume of water is drastically reduced resulting in more frequent contact to fish host (Uruku and Adikwu, 2017). Relation between climatic condition and its effect on parasite infections in fishes as observed in the present study revealed that the co-relation to mean intensity temperature, rainfall and the humidity has significant negative impact in *C. magur* and significant positive relation to abundance and mean intensity in *C. striata*. The other piscine hosts showed some co-relation to the climatic factors, but they were statistically insignificant.

The host-wise frequency of occurrence of helminths is noteworthy since some fishes were infected by more number of parasites than others. The reason why parasites are well colonized more in some fishes and less in others in the same spot could have various reasons like the life history and ecological characteristics of hosts, parasite body size and basic reproductive rate of parasites etc. (Poulin and Morand, 2000).

The observed frequency distribution indices in this study showed that the three taxa of helminths viz, cestode, nematode and acanthocephala were found during the sampling months of the whole year, and the lowest infection level observed was for *L. attenuatus* (9.93%) during pre-monsoon. The highest prevalence of cestodes [*Lytocestus attenuatus* (28.85%), *L. indicus* (45.26%), *L. filiformes* (32.52%), *L. longicollis* (31.80%)

and *Djombangia penetrans* (25.07%)] were observed during monsoon, a season which recieved more amount of rain and warmer than the pre-monsoon and post-monsoon. Similar type of observation was also noted in fish (three-spined sticklebacks) tapeworm, *Schistocephalus solidus* of Carsington Reservoir, Derbyshire, UK where it is explained that the higher prevalence during monsoon might be because of temperature effects on host immunity and increase in the water volume supporting the hypothesis that increased temperatures are beneficial for parasites (Labaude *et al*., 2015; Macnab and Barbar, 2012). The larval forms of the taxa Lytocestidae were found highest during the end of post-monsoon and onset of pre-monsoon indicating that the peak recruitment occurred during this period, giving rise in the parasite abundance and mean intensity during these seasons. A similar type of observation was also made by Chandra *et al*. (1997) who studied some aspects of association and development of *L. indicus* in *Clarias magur* and showed that the recruitment of this parasite occurs during winter period which is in concordance with our result. The development time of parasite is largely influenced by temperature, thus it can indirectly drive the intensity. In the case of nematodes (*Camallanus anabantis*, *Paraquimperia manipurensis* and *Neocamallanus singhi*) and acanthocephalans (*Pallisentis ophiocephali* and *P. indicus*) the prevalence values were observed highest during pre-monsoon. Similar type of observation was also noted by Rui *et al.* (2013) for helminth *Pallisentis* sp. The increase or decrease in the parasite population is accounted mainly to the life cycle of the parasite in relation to temperature that favours the process. The embryonated females of *C. anabantis* were seen highest during pre-monsoon and thus the mean intensity peaked during monsoon where the larval forms were recovered in great

numbers. This result is in conformity with the observation on seasonal dynamics of *Camallanus anabantis* in West Bengal (De, 1993).

In the present study, the cestodes of the family Lytocestidae were found only in *Clarias magur* but not in other fishes of the same locality. Similarly, acanthocephalans were also recovered only from channid fish. Parasites are subjected and stimulated by the same environmental factors as their hosts. However, some parasites require very specific environmental conditions to thrive and they are found only in those hosts that could provide the particular conditions may explain the host specificity of the parasites (Lafferty and Kuris, 1999).

The extent of parasite infection can be expressed as the intensity, prevalence and abundance of infection (Lohmus and Bjorklund, 2015), therefore, to know the extent of helminth infections in some selected freshwater fishes of Manipur, the present work was undertaken and it showed that the parasites are most prevalent during monsoon and pre-monsoon. Hence, control measures can take this factor into consideration and treatment provided during these seasons can be effective in controlling the helminth infections. Knowledge on occurrence, seasonality, diversity and infection indices in fishes by parsites may highlight the importance of parasite studies in controlling and executing preventive measures of fish diseases caused by parasites. The results of the present study provide rudimentary knowledge of parasite spectrum and its seasonality which will help in management to prevent infections.

Different management strategies have been suggested to prevent introduction of disease to healthy fish and also to prevent propagation of the existing disease (Faruk and Anka, 2017; Idowu *et al.,* 2017). This include drying the ponds and leaving it empty or

unoccupied for short time since fish parasites and their eggs are usually killed within a period of 3-20 hours of complete dessication. Removing of parasite from infected fishes one by one is not a feasible method. In such cases, prevention and control strategy involving different parameters depending on the occurrence of parasites are encouraged (van Duijn, 1973). Since different parasite species react differently to treatments thus, it is advisible to consult veterinarian before treatment.

SUMMARY AND CONCLUSION

In the present study, a total of two thousand five hundred thirty-three fishes belonging to ten species of different orders (Channiformes, Perciformes, Synbranchiformes, Cypriniformes, Siluriformes and Osteoglossiformes) were examined for three years i.e., August 2014 - July 2017. Out of the ten species of fishes, seven of them were found to be infected by Cestodes, Nematodes, Trematodes and Acanthocephala.

A parasitological survey in the mentioned fishes revealed the presence of twenty different types of helminth parasites. This includes six different species of nematodes (*Camallanus anabantis*, *Neocamallanus singhi*, *Paraquimperia* manipurensis, *Paracamallanus ophiocephali*, *Procamallanus* sp. and *Anisakis* sp.), six species of cestodes (*Lytocestus attenuatus*, *L. indicus*, *L. filiformis*, *L. longicollis*, *Djombangia penetrans* and *Senga lucknowensis*), four species of trematodes (*Astiotrema reniferum*, *Clinostomum philippinense*, *Phyllodistomum* sp. and *Posthodiplostomum sp.*) and four species of acanthocephalan (*Pallisentis ophiocephali*, *P. indicus*, *Pallisentis* sp. and *Echinorhynchus* sp.).

The nematode parasite *Paracamallanus* and *Camallanus* share some similar morphological features however, differences among them were sufficient enough to place them in separate genus. In the genus *Paramquimperia,* only four species have been recorded worldwide. Out of which *Paraquimperia manipurensis* represents one of them. The scanning electron microscopic study of *P. manipurensis* revealed the lip structure to

be slightly different from previously described species from this region. Thus, it is redescribed herein.

The species of the genus *Lytocestus* and *Djombangia* showed morphological resemblance with the previously described species. However, the larvae of Lytocestidae recovered during the sampling could not be identified morphologically. Thus, these larval cestode along with four other helminth parasites which could not be identified up to the species level were studied using the genetic markers namely CO1, ITS2, and 18S, and were successfully amplified. The amplicons were sequenced and submitted in the Genbank and their accession numbers acquired. The CO1 and ITS2 sequence analyses of the two metacercariae revealed that they belong to *Clinostomum philippinense* and *Phyllodistomum* sp. The 18S analyses of the two cestodes and an acanthocephalan revealed that they belong to *Lytocestus indicus, Senga lucknowensis* and *Pallisentis* sp. Additionally, ITS2 secondary morphometrics of *Clinostomum philippinense* and *Phyllodistomum* sp. depicted the hallmark four-helix model with branches, loops and bulges.

The host-wise study of prevalence, abundance and mean intensity were observed highest in pre-monsoon in case of *A. testudineus, C. striata* and *C. punctata* whereas, in *C. magur*, prevalence was highest in monsoon and abundance and mean intensity was highest in post-monsoon. The parasite wise study showed the lowest level of infection due to *L. attenuatus* (9.93%) during pre-monsoon. The highest prevalence of cestodes [*Lytocestus attenuates* (28.85%), *L. indicus* (45.26%), *L. filiformes* (32.52%), *L. longicollis* (31.80%) and *Djombangia penetrans* (25.07%)] were observed during monsoon. The larval forms of the taxa Lytocestidae were found highest during the end of

post-monsoon and on onset of pre-monsoon, indicating that the peak recruitment occurred during monsoon period, giving rise in abundance and mean intensity. In the case of nematodes (*Camallanus anabantis*, *Paraquimperia manipurensis* and *Neocamallanus singhi*) and acanthocephalans (*Pallisentis ophiocephali* and *P. indicus*) highest rate of prevalence was observed during pre-monsoon.

The spectrum of helminth parasites of piscine hosts recorded in this study reached up to twenty species. Out of which seven species (*N. singhi, P. ophiocephali, Anisakis* sp., *S. lucknowensis, C. philippinense, Phyllodistomum* sp. and *Echinorhynchus* sp.) are reported for the first time from Manipur. Moreover, stereoscan observations on the surface topography of *N. singhi, P. manipurensis, S. lucknowensis* and *C. philippinense* were carried out for the first time.

Meteorological factors like temperature, humidity and rainfall showed significant correlation with the prevalence, abundance and mean intensity of helminth infections. Temperature range of 24-27˚C revealed to be favorable for infection of helminth parasites. Therefore, we suggest that treatments should be provided during the monsoon and pre-monsoon seasons, which might prove to be effective in controlling the helminth infections. The results of the present study will help in developing comprehensive disease management strategies to control the fish-borne zoonoses and parasitic disease management in fish. Various methods have been suggested for disease management of fishes which includes pond drying, snail removal from the water bodies as they are the intermediate hosts of many cercariae, giving treatments etc.

From the present study, it is also evident that among the fish-borne helminth parasites, *Clinostomum* metacercariae and *Anisakis* sp. are the potential zoonotic trematode and nematode respectively, prevailing in Manipur, Northeast India.

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