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ORIGINAL ARTICLE

# A survey of metacercarial infections in commonly edible fish and crab hosts prevailing in Manipur, Northeast India

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**Abstract** Food-borne trematode infections, which are mainly transmitted through consumption of inadequately cooked or raw fish and crabs, affect a large section of population, particularly in Southeast Asian countries, thus eliciting a remarkable morbidity and causing serious damage to health. In India, centering in several mountainous regions of the Northeast, the natives have the habit of consuming such fish or crabs that still sustain viable infective larval stage (metacercaria) of trematode flukes in their muscle tissue. The present study was undertaken to ascertain the spectrum of metacercarial diversity in commonly edible freshwater fishes and crab species in the northeastern state of Manipur and to adjudge their zoonotic potential, if any. Commonly edible fishes belonging to 15 species from 12 localities and crabs belonging to 2 species from 11 localities across Manipur state were surveyed for the purpose. The study revealed that 3 species of fishes (*Channa punctatus*, *C. straitus* and *Wallago attu*) harboured 4 different types of metacercariae belonging to 4 trematode families—*Euclinostomum heterostomum* (Clinostomidae); *Lophosicyadiplostomum* sp. and *Posthodiplostomum* sp. (Diplostomidae); and *Polylekithum* sp. (Allocreadiidae) in addition to adult flukes of *Isoparorchis hypselobagri* (Isoparorchiidae). Among these, metacercariae of *Posthodiplostomum* showed the highest prevalence (2.33 %) though a low abundance, while for other species the prevalence ranged between 0.25 and 1.19 %. The crab species (*Barythelphusa lugubris masoniana* and *Potamiscus manipuriensis*) were found infected with 4 different types of metacercariae representing the genera *Paragonimus* (Troglotrematidae) and *Microphallus* (Microphallidae). The paragonimids showed a higher rate of occurrence (~4–25 %) compared to microphallids (~15 %). The crustaceans surveyed emerged as prospective intermediate hosts for lungflukes. Identifying the potent vectors for zoonotic parasites helps in control measures towards their transmission to higher mammals.

**Keywords** Trematode · Metacercaria · Fish · Crustacea · Zoonosis · Manipur · Northeast India

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## Introduction

Trematodiases are caused by digenetic flukes (Platyhelminthes: Trematoda) and are a major public health problem world wide. Food-borne trematodiasis (FBT) is an important group of neglected tropical diseases, which are zoonotic as they are transmitted by the consumption of raw or undercooked aquatic foods that harbour the metacercaria (i.e., infective larval stage) of the fluke. Over 100 species of FBTs are known to cause infections in humans (WHO 2009). FBTs are endemic in various parts of the world, particularly Southeast Asian regions (Dixon and Flohr 1997). As estimated, more than 750 million people are at risk of infections with food-borne trematodes (Keiser and Utzinger 2009). Fishes and crustaceans may harbour the infective metacercarial stage of a large number of trematodes, which are responsible for FBT (WHO 1995; Lun et al. 2005; Bullard and Overstreet 2008), thus serving as vector for some human helminthic diseases. In India, like most other tropical countries, parasitic infections play a major role in public health. Particularly in Manipur, Northeast India, the native people have similar food habits to those of the Southeast Asian neighbours of consuming

raw or inadequately cooked fish or crabs that still sustain viable infective stages (i.e., metacercaria) of trematode flukes in their tissues (Mahanta 1990). Thus, trematode infections of the lung and intestine are especially significant as potential zoonoses in the region.

Freshwater fish harbour infections caused by various trematode species belonging to different families viz., Phyllodistomidae, Monorchiidae, Zoogonidae, Callodistomidae, Homalometridae, Opecoelidae, Microphallidae, Opisthorchiidae, Heterophyidae, Isoparorchiidae, Clinostomatidae, Diplostomidae etc. (Yamaguti 1971). A pioneering amount of literature is available on metacercariae of various digenetic trematodes from fishes all over the world (Malek and Mobedi 2001; Arafa et al. 2005; Silva-Souza and Ludwig 2005; Vianna et al. 2005; Rim et al. 2008; Han et al. 2008; Sohn 2009; Skov et al. 2009; Sohn et al. 2009; Gustinelli et al. 2010; Thuy et al. 2010; Gholami et al. 2011), including that from India (Kumari 1994; Jhansilakshmibai and Madhavi 1997; Singh et al. 2003; Vankara et al. 2011; Shareef and Abidi 2012).

The Crustacea-borne trematode infections are caused by fluke parasites belonging to families Paragonimidae, Microphallidae, Lecithodendriidae, Brachylaimidae etc. (Yamaguti 1971; Anantaraman and Subramoniam 1976; Janardanan et al. 1987). Among these infections, *Paragonimus* spp causing paragonimiasis pose a continuing public health problem (WHO 1995; Blair et al. 1999; Nakamura-Uchiyama et al. 2002). They occur in a number of countries in several regions of Asia, Africa and Latin America (Toscano et al. 1995). In context of India, the focal transmission of paragonimiasis has been documented in north-eastern states of the country, such as Arunachal Pradesh, Manipur and Nagaland (WHO 2009). Several species of *Paragonimus* have been reported to occur in the commonly edible crab species prevailing in the mountainous ranges of Northeast India (Singh 2002, 2003; Narain et al. 2003; Singh et al. 2006, 2007, 2009; Tandon et al. 2007; Devi et al. 2010). The regions, where crabs are commonly consumed as part of their traditional cuisine are suspected foci for human infection (Tandon et al. 2007). However, there is scanty information available on themetacercarial infection status in commonly edible freshwater fishes and crabs in the Manipur region of Northeast India. So, the present study was undertaken to ascertain the

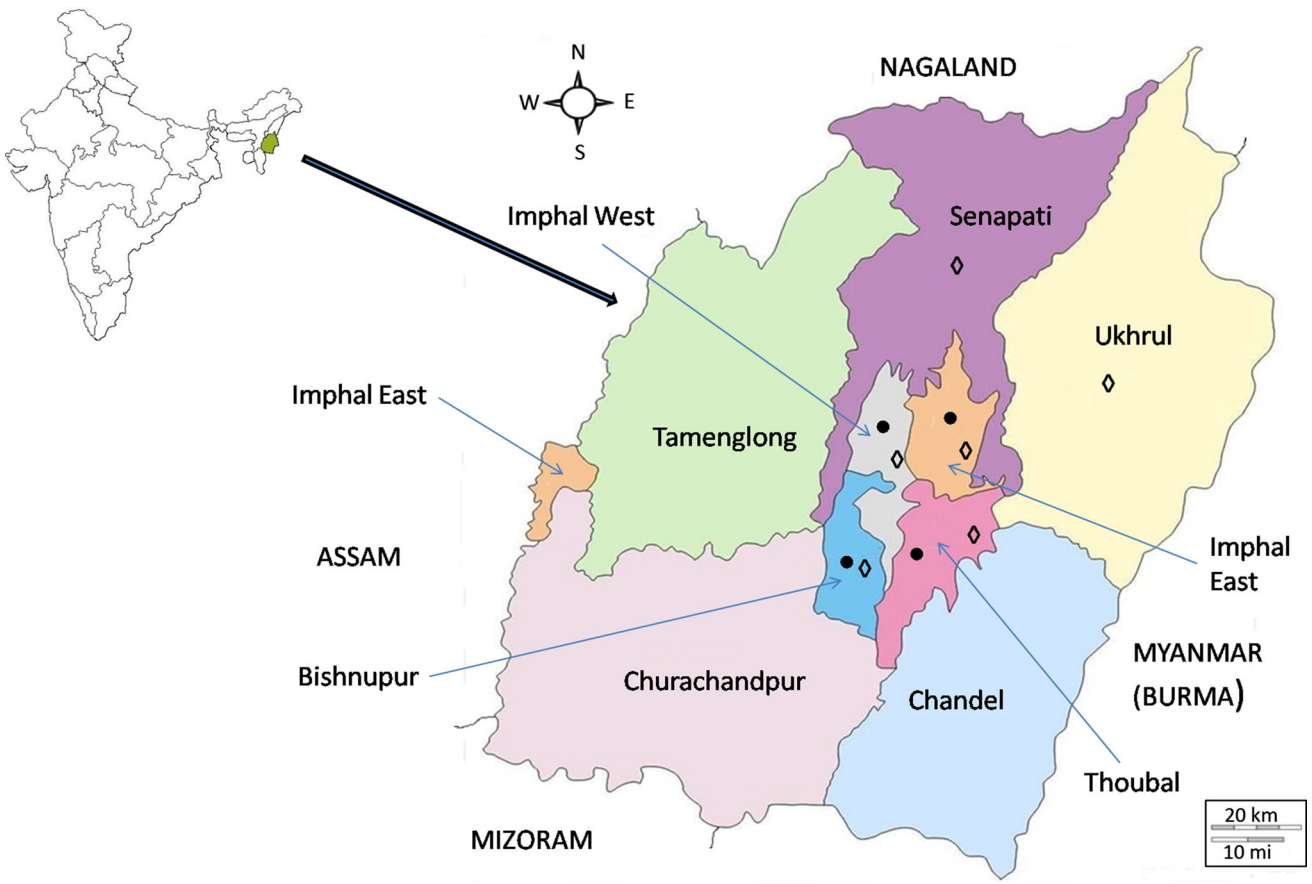


**Fig. 1** Map of Manipur depicting the different locality sites surveyed for the collection of commonly edible fish (*circle*) and crabs (*diamond*). [Maps source: http://www.himalayanfootsteps.com/destinations/india/manipur/; http://d-maps.com/carte.php?num_car=31957&lang=en]

**Table 1** Freshwater piscine hosts examined from different localities in Manipur

| Name of the host | Locality | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Lamlong | Yaralpat | Lamphel | Mayang-imphal | Sekmaijing | Tera | Wangoi | Moirang | Nambol | Oinam | Tengtha | Thoubal |
| Anabantidae | | | | | | | | | | | | |
| *Anabas testudineus* | + | + | + | – | – | – | – | + | – | + | – | + |
| Bagridae | | | | | | | | | | | | |
| *Mystis bleekeri* | – | + | – | + | – | – | – | + | – | – | – | – |
| Belontidae | | | | | | | | | | | | |
| *Colisa fasciatus* | – | – | + | – | – | – | – | – | – | – | – | – |
| Channidae | | | | | | | | | | | | |
| *Channa punctatus* | + | + | + | + | – | + | + | + | + | + | + | + |
| *Channa striata* | – | – | + | + | – | – | – | + | – | – | – | + |
| Cyprinidae | | | | | | | | | | | | |
| *Amblypharyngodon mola* | – | – | – | + | – | – | – | – | – | – | – | + |
| *Catla catla* | – | – | – | – | – | – | + | – | – | – | – | – |
| *Cirrhinus mrigala* | – | – | – | + | – | – | + | – | – | – | – | – |
| *Cyprinus carpio* | – | – | – | – | – | + | + | – | – | + | – | – |
| *Punctius jayarami* | + | – | – | + | – | – | + | + | – | + | – | – |
| Clariidae | | | | | | | | | | | | |
| *Clarius batracus* | + | – | + | – | – | – | – | – | – | – | – | – |
| Gobiidae | | | | | | | | | | | | |
| *Glossogobius giuris* | – | – | – | – | – | – | – | + | – | – | – | – |
| Heteropneustidae | | | | | | | | | | | | |
| *Heteropneustus fossilus* | – | + | + | + | + | – | + | + | + | – | – | – |
| Notopteridae | | | | | | | | | | | | |
| *Notopterus notopterus* | – | – | – | – | – | – | – | + | – | – | – | – |
| Siluridae | | | | | | | | | | | | |
| *Wallago attu* | – | – | + | – | – | – | – | – | – | – | – | – |
| Symbranchidae | | | | | | | | | | | | |
| *Monopterus cuchia* | – | – | – | – | + | – | – | – | – | – | – | – |

"+" localities where collections were made

spectrum of metacercarial diversity in these common components of the traditional cuisine in Manipur state and to adjudge their zoonotic potential, if any.

## Materials and methods

### Host collection and identification

Manipur, located in the north-eastern region of India (22°16′54″N and 88°54′39″E), has a rich variety of piscine and crab hosts. For the collection of fishes, surveys covered localities under four districts in Manipur—Imphal East, Imphal West, Bisenpur and Thoubal; crabs were collected from additional two more districts (Senapati and Uhkrul) during a 2-year period beginning 2009 (Fig. 1; Tables 1, 2). The fishes collected represented 16 species under 15 genera and 11 families. They were identified following the standard literature (Vishwanath et al. 2007). The crab hosts were collected from various localities (hill streams and paddy fields) and rural markets in Manipur. The collection comprised two species (representing two genera under two families), which were identified with the help of the Zoological Survey of India, Kolkata.

### Examination of the host for metacercariae

The gastrointestinal tract, other internal organs and muscle tissue of the fish hosts were teased in 0.7 % saline at room temperature and examined for recovery of the metacercarial and/or adult stages of the fluke parasites.

For isolation of metaceracaria from muscle tissue of the crab host, the minced tissue was digested overnight by incubating at 37 °C in artificial gastric juice following the

**Table 2** Crab hosts examined from different localities in Manipur

| Name of the host | Locality | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Luwangsangbam | Takhel | Yeingangpokpi | Chajing | Kangchup | Khurkhul | Leimaram | Bishnupur | Thoubal | Motbung | Ukhrul |
| *Barythelphusa lugubris masoniana* | + | + | + | + | – | + | + | + | + | – | + |
| *Potamiscus manipuriensis* | + | – | + | – | + | – | – | – | – | + | + |

earlier described procedure (Tandon et al. 2007). The digested material was filtered through a mesh-wire sieve, and the filterable sediment was then examined under a dissecting stereoscopic microscope for recovery of metacercariae.

The recovered metacercariae were duly processed for identification and preserved for further use.

### Light microscopy (LM)

For the purpose of whole mount preparations, encysted metacercariae were made to excyst by providing a gentle mechanical pressure and flattened between a glass slide and cover glass. Specimens fixed in 70 % ethyl alcohol were processed with suitable whole mount preparation following the standard protocol. Observations were made with the help of Leitz Ortholux-2 research microscope.

### Scanning electron microscopy (SEM)

Metacercariae were fixed in 10 % neutral buffered formalin and processed following the standard protocol for SEM (Roy and Tandon 1991). Observations were made with JSM 35CF (JEOL) and LEO 435 VP SE microscopes at electron-accelerating voltages ranging between 10 and 20 kV.

### Spectrum of metacercariae

Preliminary identification, based on morphological criteria, was done following the standard literature (Yamaguti 1971; Bray et al. 2008; Gibson et al. 2002). The prevalence, mean intensity and abundance of metacercaria infections were calculated following Bush et al. (1997).

## Results

### Metacercariae in fishes

The status of metacercarial infections prevalent in commonly edible fish hosts from various localities of the study area is shown in Table 3. A total of 1737 fishes of sixteen species (belonging to eleven families) were surveyed (Table 1). Fishes from only one family, namely Channidae, were found to be infected with metacercariae, with a low infection rate of 1.44 %. Based on morphological and morphometric criteria (Rim et al. 2008; Sohn et al. 2009), the recovered metacercariae were identified as belonging to genera—*Euclinostomum* (Rudolphi 1809) Travassos 1928 (family Clinostomidae); *Posthodiplostomum* Dubois 1936 and *Lophosicyadiplostomum* Dubois 1936 (family

**Table 3** Status of metacercarial infection in fishes surveyed from different localities in Manipur

| Name of host | Locality-numberwise of host examined | Location of parasite in host | Metacercaria recovered |
|---|---|---|---|
| *Channa punctatus* | Lamphel-101 | Body muscle | *Polylekithum* sp. & *Posthodiplostomum* sp. |
| | Mayangimphal-69 | – | – |
| | Moirang-250 | Liver | *Euclinostomum heterostomum* |
| | Nambol-60 | – | – |
| | Porompat-65 | – | – |
| | Tengtha-51 | – | – |
| | Tera-30 | – | – |
| | Thoubal-56 | – | – |
| | Wangoi-26 | – | – |
| *Channa straita* | Lamphel-3 | – | – |
| | Mayangimphal-6 | – | – |
| | Moirang-69 | Intestinal lumen | *Lophosicyadiplostomum* sp. |
| | Thoubal-6 | – | – |

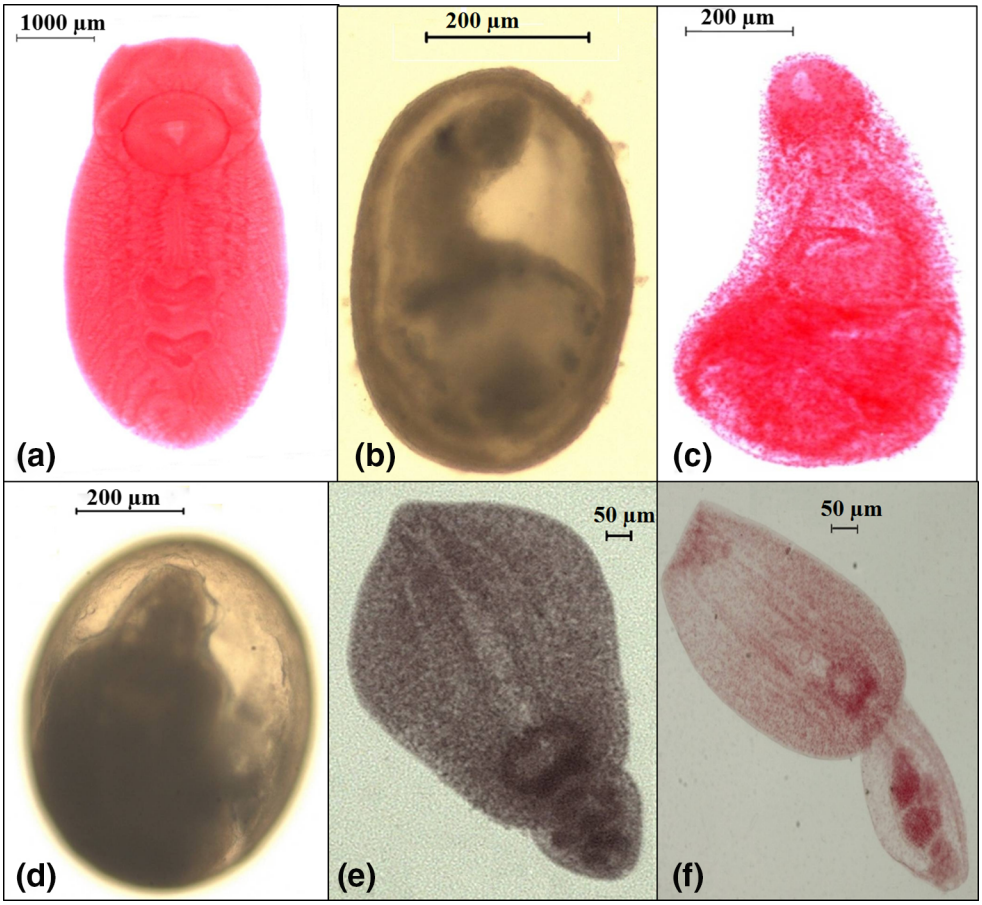


**Fig. 2** LM pictures of the encysted (**b**, **d**) and excysted (**a**, **c**, **e**, **f**) metacercariae recovered from fish hosts. **a** *Euclinostomum heterostomum*; **b**, **c** *Polylekithum* sp.; **d**, **e** *Posthodiplostomum* sp.; **f** *Lophosicyadiplostomum* sp.

**Table 4** Status of metacercarial infection in crab species surveyed from different localities in Manipur

| S. no. | Host species | Locality surveyed | No. of hosts examined | Status of metacercarial infection |
|---|---|---|---|---|
| 1. | *Barythelphusa lugubris masoniana* | Bishenpur | 10 | – |
| | | Chajing | 30 | – |
| | | Kangchup (Singda) | 16 | – |
| | | Khurkhul | 40 | – |
| | | Leimaram | 35 | – |
| | | Luwangsangbam | 20 | – |
| | | Takhel | 60 | – |
| | | Thoubal | 46 | – |
| | | Ukhrul | 16 | – |
| | | Yeingangpokpi | 326 (total = 599) | *P. westermani* |
| 2. | *Potamiscus manipuriensis* | Kangchup (Singda) | 30 | – |
| | | Luwangsangbam | 35 | – |
| | | Motbung | 175 | *Microphallus* sp. 1 and *Microphallus* sp. 2 |
| | | Ukhrul | 50 | *P. heterotremus* |
| | | Yeingangpokpi | 45 (total = 335) | – |

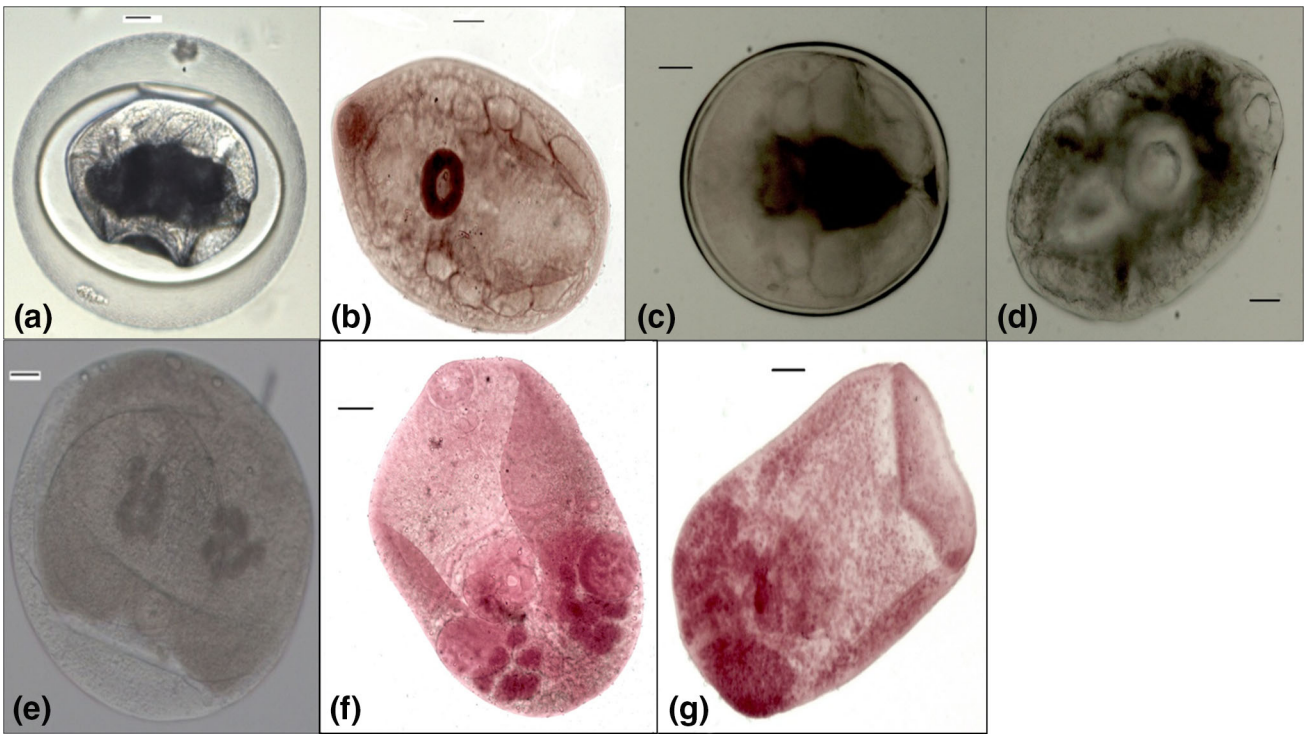


**Fig. 3** LM pictures of the encysted (**a**, **c**, **e**) and excysted (**b**, **d**, **f**, **g**) metacercariae recovered from crab hosts (*Scale bar* 50 μm). **a**, **b** *Paragonimus westermani*. **c**, **d** *Paragonimus heterotremus*. **e**, **f** *Microphallus* sp.-type 1. **g** *Microphallus* sp.-type 2

Diplostomidae) and *Polylekithum* Arnold 1934 (family Allocreadiidae), all of which are briefly described with a mention of important characters as follows (Fig. 2).

*Euclinostomum heterostomum*

Excysted metacercaria body stout; 5.50 × 2.20 mm in size; oral sucker surrounded by collar-like folds, relatively smaller than ventral; ventral sucker large, highly developed; intestinal caeca long, with lateral diverticular branching; reproductive organs well developed, testes irregular in shape; cirrus sac intertesticular; ovary small, round, intertesticular; uterus reaching forward up to ventral sucker.

*Polylekithum sp.*

Metacercarial cyst oval in shape, 0.50 × 0.35 mm in size. Excysted metacercaria elongate, 0.88–1.08 × 0.35–0.41 mm in size; body unspined; oral sucker subterminal; prepharynx short; intestinal caeca wide, reaching posterior extremity; reproductive organs under-developed; excretory bladder showing presence of granules.

*Posthodiplostomum sp.*

Metacercarial cyst oval in shape, 0.64–0.67×0.53–0.56 mm. Excysted metacercaria with distinctly bipartite body, 0.50–0.68 mm long; forebody lanceolate, 0.40–0.50× 0.32–0.57 mm, hindbody oval, 0.10–0.18 × 0.14–0.20 mm; oral and ventral suckers feebly developed; holdfast almond-shaped; testes tandem; ovary ellipsoidal, pretesticular.

*Lophosicyadiplostomum sp.*

Metacercaria nonencysted, body bipartite, 0.77–0.89 mm in length; forebody 0.52–0.54 × 0.23–0.29 mm, hindbody 0.26–0.28 × 0.16–0.18 mm; oral sucker elliptical, with equatorial muscular rings surrounding it dorsally and laterally; pseudosuckers present; ventral sucker small, located in forebody; holdfast organ circular, with central cavity; testes tandem, globular; ovary oval, pretesticular.

Metacercariae in crab hosts

The status of metacercarial infection prevalent in crab hosts (represented by *Barytelphusa lugubris masoniana* (Gecarcinucidae) and *Potamiscus manipuriensis* (Potamidae)) from the study area is shown in Table 4. Both species were found to be infected with metacercariae, with an infection rate of 28.90 %. Based on morphological and morphometric criteria the recovered metacercariae were identified as two species of the genus *Paragonimus* (family Paragonimidae) and two more types representing the genus

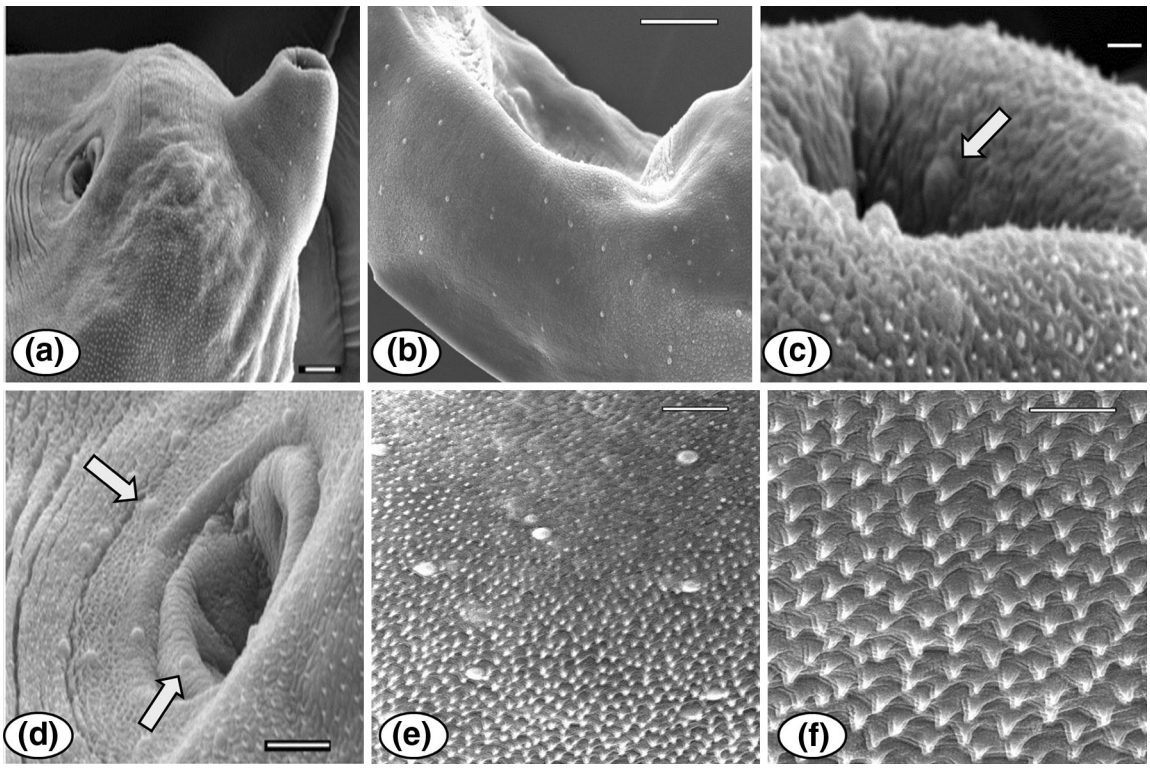


**Fig. 4** SEM views of *Paragonimus westermani* metacercaria: **a** Excysted metacercaria, showing the oral and ventral suckers, also the presence of papillae in anterior end of the body (*Scale bar* 20 μm), **b** showing the distribution of papillae on the lateral side of the body (*Scale bar* 50 μm), **c** view of the anterior region near the oral sucker, showing the presence of randomly distributed papillae (*arrow*) along with body spines (*Scale bar* 2 μm), **d** ventral sucker region, showing the presence of papillae around the sucker (*arrows*) (*Scale bar* 10 μm), **e** tegumental surface showing the single-pointed, backwardly directed spination pattern and randomly scattered papillae (*Scale bar* 10 μm), **f** tegument spination a magnified view (*Scale bar* 5 μm)

*Microphallus* (family Microphallidae) (Fig. 3), as briefly described below.

*Paragonimus westermani*

Encysted metacercaria round in shape, 0.81 mm in diameter. Excysted metacercaria 0.47–0.61 × 0.24–0.36 mm in size; whole body covered with single pointed spines and small dome shaped papillae, latter also dispersed around oral and ventral suckers (Fig. 4); oral sucker terminal; ventral sucker pre equatorial, larger than oral sucker; pharynx present, oesophagus very short; intestinal bifurcation in anterior forebody, caeca undulating terminating at posterior end; reproductive anlagen poorly developed.

*P. heterotremus*

Encysted metacercaria rounded, 0.43 mm in diameter. Excysted form 0.41–0.52 × 0.15–0.23 mm in size; single-pointed tegumental spines distributed all over body surface, pointing towards posterior side; oral sucker terminal; ventral sucker pre-equatorial, distinct, exhibiting rings of papillae; pharynx present, oesophagus very short; intestinal bifurcation at anterior forebody, caeca undulating, terminating at posterior end; reproductive organs not yet developed (Fig. 5).

*Microphallus spp*

Metacercaria *Microphallus*-type 1: cyst form rounded, thin-walled, 0.83 mm in diameter. Excysted form having globular body, 0.42–0.52 × 0.37–0.50 mm in size; ventral sucker post equatorial; oesophagus long; caeca short, divergent just anterior to ventral sucker; testes postovarian, symmetrical; ovary dextral to ventral sucker, vitellarian clusters present in groups of 6–7, posterior to each testis.

Metacercaria *Microphallus*-type 2: excysted form elongated, 0.46–0.54 mm in length, 0.20–0.24 mm in width; thick muscular structure in anterior body surrounding oral sucker; ventral sucker large-sized; ovary dextral to ventral sucker; testes postovarian, cylindrical in shape, arranged on either posteriolateral side of body.

The host fish *Channa punctatus* was found to harbour 3 metacercarial types, whereas *C. striatus* was found infected

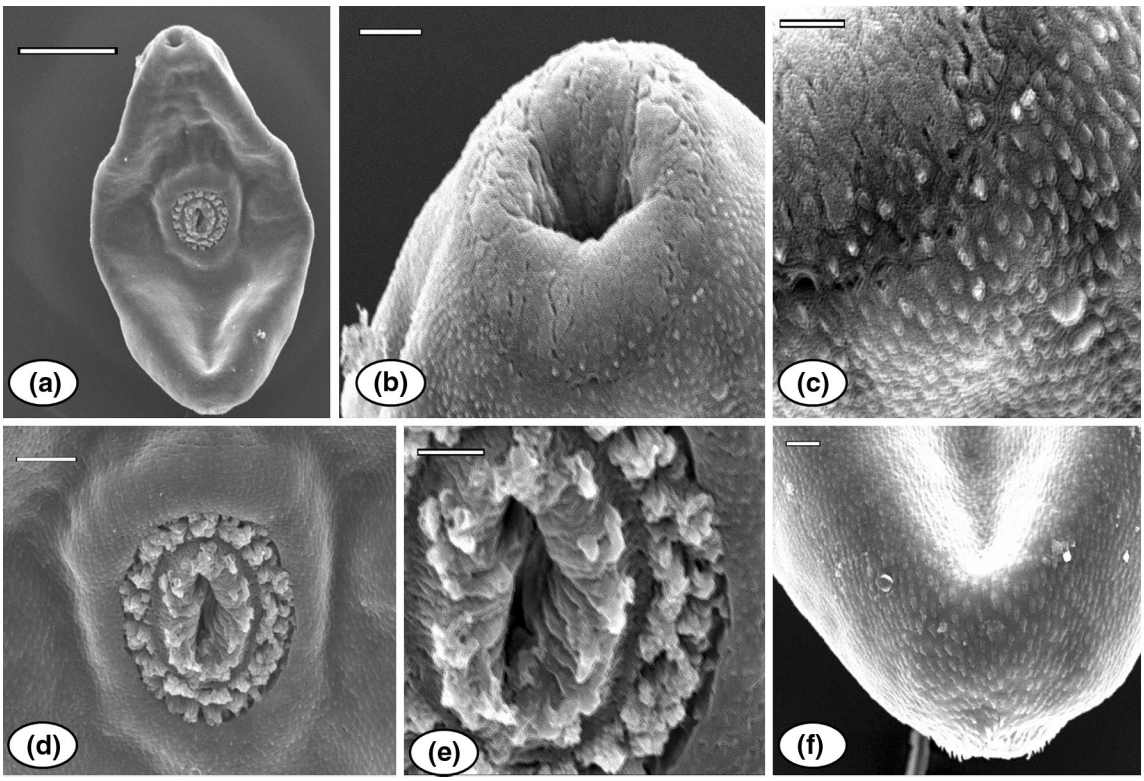


**Fig. 5** SEM of *P. heterotremus* metacercaria: **a** Excysted metacercaria, showing the oral and ventral suckers (*Scale bar* 100 μm), **b** the oral end in a magnified view (*Scale bar* 10 μm); **c** tegumental spination in anterior region (*Scale bar* 5 μm); (**d**, **e**) ventral sucker, showing circum-sucker rows of papillae (*Scale bar* 20 and 10 μm), (**f**) posterior end of the body, showing the backwardly directed spination pattern (*Scale bar* 10 μm)

**Table 5** Spectrum of metacercarial infections in the commonly edible fish and crabs species in the respective foci

| Host species surveyed | Metacercaria | Location | No. of host | | Prevalence (%) | No. of metacercaria recovered per host | Mean intensity | Abundance |
|---|---|---|---|---|---|---|---|---|
| | | | Examined | Infected | | | | |
| Fishes | | | | | | | | |
| *Channa punctatus* | *Euclinostomum heterostomum* | Liver | 771 | 2 | 0.25 | 1 | – | – |
| | *Polylekithum* sp. | Muscle | 771 | 4 | 0.51 | 1–3 | 2.25 | 0.01 |
| | *Posthodiplostomum* sp. | Muscle | 771 | 18 | 2.33 | 2–30 | 11.50 | 0.27 |
| *Channa straita* | *Lophosicyadiplostomum* sp. | Intestine | 84 | 1 | 1.19 | 6 | 6.00 | 0.08 |
| Crabs | | | | | | | | |
| *Barythelphusa lugubris masoniana* | *Paragonimus westermani* | Muscle | 599 | 150 | 25.04 | 5–20 | 6.00 | 1.50 |
| *Potamiscus manipuriensis* | *Paragonimus heterotremus* | Muscle | 335 | 15 | 4.47 | 1–3 | 1.67 | 0.08 |
| | *Microphallus* sp. 1 | Muscle | 335 | 55 | 16.41 | 2–7 | 3.27 | 0.54 |
| | *Microphallus* sp. 2 | Muscle | 335 | 50 | 14.92 | 1–3 | 0.48 | 0.08 |

with only one metacercarial type. All metacercarial cysts were recovered from the muscle tissue except for *Lophosicyadiplostomum* sp., which was found in the intestinal lumen (Table 3). *Posthodiplostomum* sp. showed the highest prevalence (2.33 %), though a low abundance (0.27) and *Euclinostomum heterostomum*, the lowest prevalence (0.25 %); for other species the prevalence ranged between 0.51 and 1.19 % (Table 5; Fig. 6a).

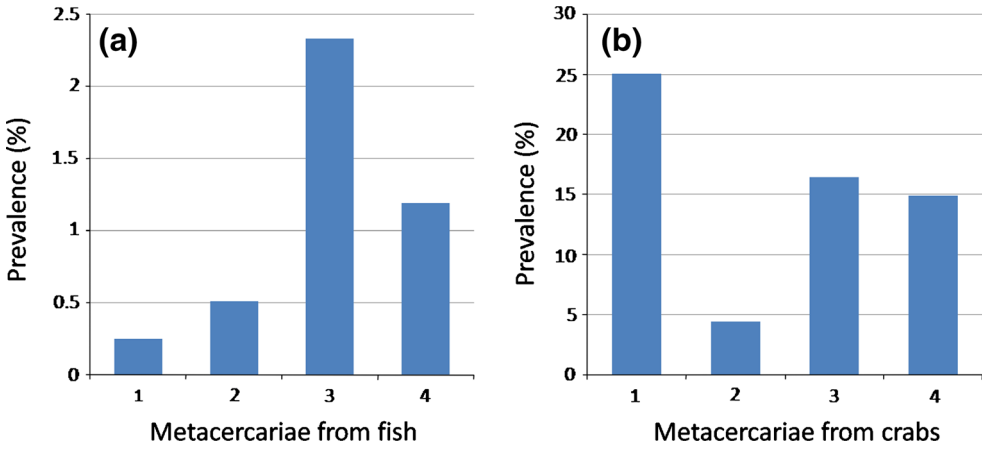


**Fig. 6** Prevalence of metcercariae in **a** fish hosts: 1, *Euclinostomum heterostomum*; 2, *Polylekithum* sp.; 3, *Posthodiplostomum* sp.; 4, *Lophosicyadiplostomum* sp. **b** crab hosts: 1, *Paragonimus westermani*; 2, *P. heterotremus*; 3, *Microphallus* sp.-type 1; 4, *Microphallus* sp.-type 2

Among the paragonimids, *Paragonimus westermani* showed a higher prevalence (25.04 %) and abundance (1.50) compared to *P. heterotremus*. The microphallids also showed a prevalence ranging between 15 and 16 % (Table 5; Fig. 6b).

## Discussion

As per the reports of WHO fish-borne trematode zoonoses are a serious health hazard (WHO 1995) and the subject has been recently reviewed (Chai et al. 2005). In the present study, among all the fish species studied, only the members of the family Channidae were found to harbour metacercariae. The channid fishes are reported to harbour metacercariae of several trematodes, e.g. *Atrophecaecum hindusthanensis*, *Clinostomum* sp., *E. heterostomum*, *Diplostomulum cerebralis*, *Neascus gussevi*, *Metaclinostomum srivastavi* and *Tetracotyl szidati*, to name a few (Chakrabarti 1974; Jhansilakshmibai and Madhavi 1997; Thapa et al. 2008; Vankara et al. 2011). Several species of *Euclinostomum* have been reported from India; these include *E. indicum* and *E. heptacaecum* from *C. punctatus* and *E. channi* from *C. marulius* (Bhalerao 1942; Jaiswal 1957). In the present study *E. heterostomum*, found encysted in the liver of *C. punctatus*, showed a low prevalence (0.25 %). In a similar study carried out in Meghalaya (another state in northeast India), *C. striatus* and *C. punctatus* also showed a low prevalence of *E. heterostomum* (1.44 and 0.74 %, respectively) (Thapa et al. 2008). Metacercarial infection of *Clinostomum* sp. that causes considerable damage to the viscera and muscles of many fish species has been reported from *C. punctatus* and *Heteropneustus fossilis* (Kalantan et al. 1987; Thapa et al. 2008; Vankara et al. 2011; Shareef and Abidi 2012). Besides affecting the nutritional value and/or mortality rates of the infected fish, infections by metacercariae of *Clinostomum* species are also important as potential fish-borne zoonoses (Kamo et al. 1962; Chung et al. 1995; Kitagawa et al. 2003; Dzikowski et al. 2004; Park et al. 2009). These parasites have been reported to cause laryngopharyngitis or even asphyxia and ocular parasitosis in human subjects (Eiras 1994; Tiewchaloern et al. 1999). However, in the present study, the *Clinostomum* infection was not detected in the fishes examined, though *C. punctatus* was found infected with three other types of metacercariae. Species of *Polylekithum* (= *Procreadium*) are known to occur in cyprinid fishes and have been reported from birds also ( Verma 1936; Vidyarthi 1938; Jaiswal 1957; Kakaji 1969). It is for the first time that *Polylekithum* sp. infection has been reported from *C. punctatus* through the present study. The same host also harboured the metacercaria of *Posthodiplostomum* sp. as coinfection with *Polylekithum* sp. Many species of *Posthodiplostomum* (namely *P. austral*, *P. oblongum*, *P. opisthosicye*, *P. botauri*, *P. grayii* and *P. milvi*) have been reported from birds (Dubois 1937, 1969; Vidyarthi 1938; Verma 1936; Fotedar and Raina 1965); a few reports are there from crucian carp and common carp (Ishii 1951; Nagasawa et al. 1989) and from *Channa argus* as well (Nguyen et al. 2012). *Channa striata* also harbours metacercariae of *Haplorchis* sp., *Clinostomum complanatum* and *E. heterostomum* (Chakrabarti 1974; Thapa et al. 2008). In the present study, *C. striata* was found to be infected with the metacercaria of *Lophosicyadiplostomum* (family Diplostomidae)—the first report from the region.

As in the present study, natural infections of *P. westermani* metacercariae have been reported in *B. masoniana* in several foci of Arunachal Pradesh (North-east India) (Tandon et al. 2007; Devi et al. 2010). *Potamiscus manipurensis, Alcomon superciliosum* and *Barytelphusa lugubris* are identified as common potential second intermediate hosts for other *Paragonimus* species as well including *P. heterotremus, P. hueit'ungensis, and P. skrjabini* in Northeast India (Singh and Singh 1997; Singh 2002, 2003; Singh et al. 2006, 2007, 2009, 2012). In India *P. westermani* has been reported from various carnivorous

mammalian hosts such palm civet cat, domestic dogs, panther, cat, tiger and mongoose (Rao 1935; Srivastava 1938; Dutta and Gupta 1978; Singh and Somvanshi 1978; Gaur et al. 1980; Parihar and Shrivastava 1988; Blair et al. 1999). An epidemiological survey carried out (during 1980s) in the Manipur region revealed the prevalence of paragonimiasis in human subjects in the region (Singh et al. 1993). In the present survey, *P. manipuriensis*, also harboured other metacercariae of the microphallid trematode genus—*Microphallus*, beside *P. heterotremus*. Metacercarial stages of *Microphallus* have been earlier reported from sand crabs and brackish-water prawns (Anantaraman and Subramoniam 1976; Jayasree et al. 2001). Metacercariae of *Microphallus indicus* have been reported from *B. lugubris* in Meghalaya (Goswami et al. 2013). However, the two microphallid metacercaria types recovered during the present study were morphologically very different from *M. indicus*. So far, from India only a few microphallid taxa have been identified, namely *Basantisia ramai*, *Levinseniella indica* and *Pseudospeloterma indicum* from birds (Lal 1936; Pande 1938; Murhar 1960), *Mehraformes jabalpurensis* and *M. indicus* from reptiles (Bharadwaj 1963; Mukherjee and Ghosh 1967), *Megalatriotrema hispidum* from the common frog (Rao 1969), and *Spelotrema narii* from jackals (Rao 1965).

The overall prevalence of metacercarial infections in the crustacean hosts was found to be optimally high (35.8 %). Among the crab species surveyed, *Barythelphusa* emerged as the potent vector and transmitter host for *P. westermani*, a high prevalence (25.04 %) of which was recorded in the region. Paragonimiasis caused by *P. westermani* is one of the medically important food-borne trematodiases in the tropical, subtropical, and some temperate countries (Miyazaki 1991; Blair et al. 1999).

As evident from the foregoing account, amongst the metacercarial infections prevalent in the region only two—*Clinostomum* sp. occurring in channid fishes, and *Paragonimus* spp in crustacean hosts—are known to be having zoonotic implications. However, *Clinostomum* infections were not encountered in the present study. Therefore, in view of prevalent culinary practices and food habits of the natives, paragonimiasis emerged as the only (crab-borne) potential zoonosis and warrants a thorough epidemiological study in suspected focal areas of infection in the region.

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