**Review Paper:**

**Freshwater larval digenetic trematode parasites in India: an epitomised review**

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**Abstract**

It is well documented that various developmental larval stages of digenetic trematode parasites of vertebrates including humans and domestic animals such as sporocyst, redia and cercaria generally develop in their intermediate aquatic snail hosts. In India, both common and rare forms of these trematode larvae have been reported from diverse freshwater snail species inhabiting various lentic and lotic habitats of different geographical provinces. However, most of the studies have been conducted so far on their taxonomy, seasonal occurrence or infection and behaviors.

Nevertheless, some excellent and original research works on their certain biology such as mode of feeding (nutrition), digestion, neuroanatomy, histopathology, parasitic castration, host-specificity etc. have also been done. Besides these studies, for the first time in-vitro culture up to juvenile stage of trematode larvae has also been successfully done in India. In the present communication, the basic and significant research works done so far on biology of freshwater larval trematode parasites in India have been briefly and critically reviewed.

**Keywords:** Biology, Freshwater, Histopathogenesis, Larval trematodes, Parasitism, Snail hosts, India.

**Introduction**

Most of the digenetic trematode parasites cause several dreaded trematodiasis such as schistosomiasis, fascioliasis, amphibiliasis, echinostomiasis etc. in humans, livestock and in other vertebrates. But their different larval stages viz. sporocysts, rediae and cercariae are also pathogens for their aquatic intermediate snail hosts. Due to variation in morphology, behavior and pathogeneity, the larval trematodes attracted attention of many helmintologists to reveal their mysteries.

Snails (Mollusca; Gastropoda) are unsegmented coelomate invertebrate animals that are found in all kind of aquatic habitats and feed on decaying organic matters as well as the planktons. Because of their feeding habits, majority of freshwater snail species such as Lymnaea acuminata f. patula, L. acuminata f. chilamys, L. acuminata f. typica, L. acuminata f. rufescens, L. luteola f. australis, L. luteola f. typica, L. luteola f. impura, Planorbis (Indoplanorbis) exustus, Faunus ater, Melania (Ploita) scabra, Thiara (Tarebia) lineata, Melanoides striatella tuberculata, Vivipara bengalis race giganticus and V. bengalis race mandiensis act as most common intermediate hosts of digenetic trematode parasites of diverse vertebrates. Indeed, these freshwater snails complete the life cycle of these trematode parasites and harbor their asexual reproductive stages, sporocyst, redia and cercaria and rarely metacercariae.

It is needless to emphasize the importance of ecological studies on the aquatic snails for their role in the classification of freshwater lentic and lotic habitats as well as the vectors which spread well known pathogens causing dreaded trematodiasis in man and animals. The presence of snail species in any geographical region also indicates the possibility of spreading of particular trematodiasis.

Snails serve as hosts for at least two stages in the life cycle of digenetic trematodes. As soon as the miracidium hatches from the eggs, it penetrates the soft tissues of snails to be transformed into the next larval stage called sporocyst. The latter gives rise either to daughter sporocysts or rediae depending on the developmental pattern of that species. Different kinds of sporocysts have been reported that either produce cercariae directly viz. sporocyst of xiphidium cercariae and certain furcocercous species while others give rise to rediae which in turn produce a large number of cercariae viz. monostome, gyrocoepalhus, echinostome and amphistome.

In India, the research works that have been conducted so far on larval trematodes such as taxonomy of diverse cercariae and their seasonal occurrence and swimming behavior, mode of feeding or nutrition, digestion mechanism, neuroanatomy (nervous system), histopathogenesis in vital organs such as digestive gland (haeapatopancreas) and gonads (parasitic castration), in-vitro culture up to juvenile stage, etc. are briefly and critically reviewed and also identified the possible future research scopes for the understanding of basic biology of these trematode larvae.

**Diverse cercariae:** Numerous forms of larval trematodes have been reported time to time from different geographical regions which are well documented. But in India, very limited reports from different provinces on freshwater larval trematodes are available. However, based on available published reports in India, the most common cercariae...
(Figures 1a-e) are monostome, xiphidio, furcocercous, amphistome, echinostome and gymnocephalous recovered from various freshwater snails species. Rare forms of sporocyst and cercariae have also been reported. In certain snail species, few metacercariae have also been recovered. Most of the helminthologists classified various cercariae based on their morphological features and flame cell formulae. But in this way there are more chances of duplication or wrong identification and classification of cercariae having almost similar morphological features and flame cell formula.

**Figures 1a-e:** Cercariae and metacercariae recovered from snail hosts (a) *V. bengalensis*, (b) *M. tuberculata*, (c) *I. exustus* and *G. convexiusculus*, (d) *L. luteola* and (e) *L. acuminate*. 

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1-3,5,7,1,15,17,21,31,58,61,66-67,69,71,75,77-83,86-87,89-91
27,62,68,70
9,37,65
To avoid such errors, the most ideal method for classification and identification of variety of cercarial species is DNA analysis or karyotyping. Molecular approaches for sensitive and specific detection of the parasite species in the snail hosts are not being widely used in India.

Usually, snails are infected by one type or species of cercariae at a time. However, in some cases the snail shed two types of cercariae exhibiting double infections. Such patterns of double infection in different snail species have also been reported in India.9,42,60 Other workers9,10 also found a species of cercariae that cysts with another cercariae in the same snail host species. No case of triple infection has been reported so far from India. Whatsoever, the pattern of double infection between various larval digenean species is much more governed by selection mechanism exhibited by free swimming miracidium larva and the host- specificity.

Seasonal emergence of cercarial larvae: In India, the seasonal emergence (infection) of different types of cercariae has been well studied22,32,41,43,57,85. Except amphistome, gymnocephalous and furcocercous cercariae and other forms of cercariae reveal irregular seasonal occurrence and most of them are released throughout the year from their snail hosts9. But amphistome, gymnocephalous and furcocercous cercariae follow a definite seasonal occurrence and emerge in the rainy and winter season (Figure 2).

The variation in the seasonal emergence of cercariae is much more dependent on the location of final vertebrate hosts, availability and population of intermediate snail hosts, chances of contact of free swimming miracidium larva to snail hosts, dwelling habit and behavior of snails and annual rain fall. Those trematode parasites that infect the terrestrial hosts need rain, as rain is the only ideal medium by which contaminated faecal matter with trematode fertilized eggs can reach to aquatic snail hosts.

Hence amphistome, gymnocphalous and furcucercous cercariae are generally found in rainy season. Information about these seasonal emergence of cercariae is much more useful in the management and control of trematodiases. Therefore, more such studies are needed in rural areas where domestic animals are more prevalent.

Swimming behavior of cercariae: In general, most of the cercarial species are found to be active swimmers, phototrophic and negative geotactic. Active swimmer cercariae are morphologically well adapted having streaming lined light body, spinosed body and tail, long prominent tail with fin-folds or furcal rami as found in monostome and furcocercous cercariae as compared to heavy aspinosed bodies species such as echinostome, amphistome, gymnocephalous and xiphidiocercariae.

Active swimming cercariae are generally found to be strongly negative geotaxis as compared to amphistome, echinostome, xiphidio and gymnocephalous cercariae who are passive swimmers. Active swimmers after leading few hours of swimming life sink down to the bottom and then finally embrace the death. Passive swimmers on the contrary encysted after few minutes of their emergence and await their ingestion by the final host18,65,66. The life span of passive swimmers is relatively very short as compared to their counter parts.

Figure 2: Graph showing seasonal cercarial occurrence or infection (%) in freshwater snails18.
Those cercarial species that exhibit pigmented eye spots on their bodies such as in many monostome, amphistome and few furcocercous cercariae are strongly phototropic and swim or move towards the best illuminated region. These species also change their direction when light source is moved to other side. These species are not affected by different colored light but they prefer the bright light. In India, more studies on the emergence and behaviors of diverse cercariae in relation to dwelling behavior of snail hosts as well as on various environmental factors like temperature, pH, water current etc. are needed to help in understanding the cercarial behaviors.

**Host-specificity in cercariae:** Many cercariae have been found to infect more than one snail species or have wide range of snail hosts (Table 1). But few cercariae species like monostome and transversotrematic cercariae are restricted to a particular snail species. The exact reason behind the wide and narrow range of host-specificity in cercariae is yet not clear. However, the possible reasons are the selection mechanism exhibited by free swimming miracidium larva and feeding behavior of the final host. Part of the ecology of the intermediate hosts has also role to determine the host-specificity. Infection with the larval forms is dependent on the genotype of molluscan host, as snails of different genera that share a common gene pool are prone to be infected by similar larval digeneans while differences in genes of the host snails of the same family appear to be responsible for the difference in larval population.

However, in India, research works on host-specificity of freshwater diverse larval trematode parasites are too scanty. Therefore, more work is needed on this aspect. Such studies are also useful in the mitigation or control of trematodiasis diseases in man and animals.

**Mode of nutrition and digestion:** Among intra- molluscan larval trematodes, both rediae and cercariae possess digestive system. In redia larva, digestive system consists of mouth surrounded by oral sucker followed by muscular pharynx and elongated large sized irregular single gut containing fine cellular debris. In contrast, gut of cercarial larva is bifurcated at the middle region of body into intestinal caeca which lacks food debris. In sporocyst larva, digestive system has never been reported.

For multiplication, the larval trematodes require more nutrition for their growth and production of the next generations, particularly sporocyst and redial stages. These larvae obtain nutrition directly by ingestion (for rediae) or indirectly via hydrolyzing food contents in simple form prior to absorption (for sporocysts). It is a fact that these larvae take nutrition from their surrounding i.e. digestive gland of snail which acts as the pool of nutrition. Depletion of nutrients in host tissues (digestive gland) due to infection has also been proved histochemically. However, certain chemicals secreted either by parasites or partly by host cells for hydrolyzing the food components prior to absorption are nothing but enzymes. This accounts for increased enzyme concentration in the infected digestive gland of the snail host. Depletion of food components and increase in the concentration of enzymes are circumstantial evidences of infected digestive gland.

The mode of nutrition and digestion in these larvae has been well studied by histochemical localization of food nutrients as well as digestive enzymes or hydrolases. Based on the degree of activity of these biochemistry in the infected and non-infected digestive gland of snail as well as in trematode larvae, it has been concluded that rediae generally use both routes tegument and caecal gut to obtain the nutrition whereas cercariae use is evidenced by histochemistry only the tegument.

This indicates the former to be nutritionally advanced over the latter. Although, the mode of nutrition and digestion mechanism in sporocyst larvae is yet not clear. However, authors believe that these larvae also utilize the tegument route (extra-cellular digestion) as these larvae lack gut. Nevertheless, more histochemical studies are highly needed for understanding of exact mechanism involved in the nutrition and digestion process in these larvae.

**Neuroanatomy (nervous system):** The neuroanatomy of adult digenean trematode parasites as well as their larval stages sporocyst, redia, cercaria and rarely in metacercaria has been well studied by histochemical localization of acetyl cholinesterase and non-specific esterase.

The neuroanatomy of sporocysts and rediae is not extensively studied. However, in few studies, the nervous system of sporocyst, redia, cercaria and metacercaria (Figures 3a-d) has been well described. In the sporocyst, the nervous system is very simple and under developed and composed of only a mass of cerebral complex at the anterior region of the body (Figure 3a). But in redia, a pair of nerves originates from the cerebral complex, proceeds posterior and unites to form a ganglion in the middle region of the body. From this ganglion, again a pair of fine nerves runs to posterior end of the body (Figure 3b). Cerebral complex also gives rise to 2-3 nerve branches which innervate the pharynx. But these studies are inadequate and do not fully justify the neuroanatomy of all kinds of sporocysts and rediae. To know the basic structure of nervous system of these trematode larvae, more studies are highly suggestive.

In the body of cercarial and metacercarial larvae, the basic architecture of nervous system is almost the same and is also basically similar to their adult forms. Their nervous system is composed of two cerebral ganglion connected to each other by transverse commissure to form a mass of cerebral complex and lye immediately posterior to oral sucker, three pairs of anterior and three pairs of posterior longitudinal nerve trunk that arise from the lateral sides of each cerebral ganglia (Figures 3c and d).
Table 1
Habit and habitats of freshwater snail species and recovered their cercarial and metacercarial trematode larvae

<table>
<thead>
<tr>
<th>Snail species</th>
<th>Habit/ habitat</th>
<th>Cercariae</th>
<th>Metacercariae</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>M</td>
<td>G</td>
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<tr>
<td><strong>Gastropoda Hydrobidae</strong></td>
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<tr>
<td>Bithynia stenothyroides</td>
<td>bd/o</td>
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<tr>
<td><strong>Viviparidae</strong></td>
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<tr>
<td>Vivipara bengalensis</td>
<td>bd/e</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Vivipara dissimillis</td>
<td>bd/o</td>
<td>-</td>
<td>-</td>
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<tr>
<td><strong>Thiaridae(Melanidae)</strong></td>
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<tr>
<td>Melania tuberculata</td>
<td>bd/e</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Thiara (Thiara) scabra</td>
<td>bd/o</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Faunus ater</td>
<td>bd/o</td>
<td>-</td>
<td>-</td>
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<tr>
<td><strong>Lymnaeidae</strong></td>
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<tr>
<td>Lymnaea acuminata</td>
<td>sd/e</td>
<td>-</td>
<td>+</td>
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<td>Lymnaea lateola</td>
<td>sd/e</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Lymnaea auricularia</td>
<td>sd/e</td>
<td>-</td>
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<tr>
<td>Lymnaea pinguis</td>
<td>sd/e</td>
<td>-</td>
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<tr>
<td><strong>Planorbidae</strong></td>
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<tr>
<td>Indoplanorbis exustus</td>
<td>sd/e,o</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Gyraulus convexiusculus</td>
<td>sd/e</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Abbreviations: + infected; - non-infected; bd, bottom dweller; sd, surface dweller; e, lentic (stagnant water); o, lotic (running water); A, amphistome; As, aspidogaster; E, echinostome; F, furcocercous cercaria; G, gymnocephalous; M, monostome; OP, opisthorchiid; P, plagiorchiid; S, strigeoid and X, xiphidio cercariae18,22.

Figures 3a-d: Neuroanatomy of (a) sporosyst, (b) reidia, (c) furcocercous cercaria and (d) strigeoid metacercaria *(Tetracotyle lymnaei)*19.

Abbreviations: AG, adhesive gland; AN, anterior nerves; ANC, anterior nerve cords; ARB, anterior region of body; CC, cerebral complex; EMC, immature cercaria; EP, excretory pore; FB, fore-body; FG, fore-body gland; GB, germ balls; HB, hind-body; L, leppets; MC, mature cercaria; OS, oral sucker; PDNC, posterior dorsal nerve cord; Ph, pharynx; PLNC, posterior lateral nerve cord; PNC, posterior nerve cord; PRB, posterior region of body; T, tail; TC, transverse connections; VS, ventral sucker.
Three pairs of cords can be differentiated according to their position, lateral, ventral and dorsal in the anterior and posterior regions respectively. The anterior dorsal, ventral and lateral nerves and their numerous fine nerve branches encircle and innervate the various organs or organ systems.

Most of the helminthologists have described the nervous systems mainly in the cercarial body but the actual arrangement of nerves in the tail region has not been defined properly. However, for the first time trace out the fine arrangement of nerves in the tail region. Two ganglia-one is located at the anterior end and other at the posterior end of the tail stem. From anterior ganglion a pair of nerves, dorsal and ventral proceed to posterior region of the tail where it joins the posterior ganglion. Further, both nerves are connected by number of ring shaped transverse commissures. But in furcocercous cercariae, two pairs of nerves again originate from the posterior ganglion of the tail stem to enter into each lateral side of furcal rami. These nerves in the furcal rami are also connected by number of transverse commissures.

Pathogenesis: Larval trematodes are well known for causing a great harm to their snail hosts. The larval digeneans multiply in snail hosts and the huge population produced meets its energy requirements from the reserve food of the host tissues. This leads to various types of pathologies ranging from debilitation to gigantism of the snail hosts. These larvae generally infect the vital organs such as digestive gland (haepatopancreas) and gonads (ovotestis) of snail hosts where these cause a varying degree of histopathological changes. These histological changes are very much associated with degree of parasitemia and size of trematode larvae. In severe infection, these physiologically active organs are completely destructed by autolysis and/or necrosis.

Histopathological changes (a) In infected digestive gland: After the entrance of miracidium larvae into the snail’s body, they ultimately reach to nutrient rich digestive gland where they undergo multiplication into different developmental larval stages, sporocysts, redia and cercaria. Healthy or uninfected digestive gland of the snail is basically composed of numerous ovals to elongated digestive tubules surrounded by single layer of epithelial cells on a prominent basement membrane. Generally, these tubules are compactly arranged (Figure 4a).

The infected digestive gland with trematode larvae can be identified visually as it appears brownish in color relatively in large size and friable. These larvae generally occupy the inter digestive gland tubular space (Figure 4a) but not invade tubules. However, in rare cases, it has also been observed that tubules had massive infection with metacercariae (Figures 4b and c). Basically two types of damages, mechanical and physiological occur in the digestive gland. In mechanical damages, various degenerating changes such as rupture of tunica propria, reduction in diameter and number and irregular shape of tubules and destruction and blocking of individual tubules are evident (Figure 4b). Physiological damage is generally characterized with loss of tissue integrity and necrosis of epithelial cells.

The mechanical damages of the digestive tubules appear to be the cumulative effect of the larval migration, feeding, growth and their multiplication. The physiological changes as autolysis and/or necrosis are possibly the result of the release of proteolytic enzymes from the ruptured digestive cells/ or enzymatic secretion and metabolites from trematode larvae. Due to squeezing of digestive tubules at different loci, no food is able to pass into the tubules. Therefore, there is a possibility of autolysis of epithelial cells of the tubules. Hence, degeneration of digestive tubules was generally found in severe infection.

Another possible reason for reduction in size of epithelial cells and increasing of inter and intra-tubular spaces of glands is reduction in the amount of stored nutrients and energy due to increasing demands of the developing host gonads as well as developing or multiplying parthenitae. Redial stages cause more mechanical and physiological damage as compared to sporocysts because of their mouth and locomotory organs while their pharyngeal glands and gut contribute for the physiological damages.

It has also been observed that rediae engulf the host’s digestive cells and utilize the hydrolases for their extra cellular digestion. Thus, it may be conjectured that redial stages are more hostile for the host tissues. Other pathobiological contributory factors can be parasitic secretions and excretory products that produce toxic effects.

(b) In infected gonads: The mechanism of entrance of trematode larva in gonads (ovotestis) of snail host is still unclear. However, histopathogenesis in gonads infected with trematode larva is well studied. But in India, such studies are rarely performed. These studies indicate that these larvae are more efficient and potential to destroy the gonads or check the vital gametogenesis process. This histopathological degeneration of gonads is generally referred as ‘parasitic castration’.

In hermaphroditic snails, the gonads comprise of numerous acini containing male and female germinal cells in a composite structure called ovotestis. The spaces between these acini are generally occupied by the parthenitae. The physical or mechanical damages in acini/gonads are generally associated with mild to moderate parasitemia caused by the cumulative effect of larval feeding, growth, migration, and multiplication. In severe infection, all acini are degenerated due to the hydrolytic enzymes produced by the trematode larvae which procure energy for multiplication and growth and thereby cause lysis of acinar cells.
From the ecological and evolutionary point of view, the parasitic castration in snails caused by larval trematode infection has great significance in control and balance between population sizes indifferent snail species inhabiting the same aquatic ecosystem. Whether regeneration of damaged both gonads and digestive gland of snail due to parasitism is possible or not, more such studies are highly suggestive.

Figures 4a-c: (a) Section of healthy digestive gland of *L. acuminate* (H and E x 400) (b) Section of severely infected digestive gland with double infection of cercariae and metacercariae showing disorganization (H and E x 400) and (c) Severely infected digestive gland showing autolytic necrosis (H and E x 100).33

**Abbreviations:** ADGT, atropic digestive gland tubule; AN, autolytic necrosis; C, cercaria; CEC, columnar epithelial cells; CT, connective tissue; DGT, digestive gland tubule; L, lumen; MC, metacercaria; R, redia.
Figures 5 a-d: A section of healthy gonads or ovotestis of _M. tuberculata_ snail showing various stages of gametogenesis in acini and connected to each other by connective tissues (Fig. a, H and E x 400). Section of infected ovotestis (mild infection) with trematode larvae showing degenerative changes in acini (Fig. b, H and E x 100). Moderate infection of larvae and degenerative changes in acini (Fig. c, H and E x 100). All acini completely replaced by trematode larvae (severe infection), a complete parasitic castration of gonads (Fig. d, H and E x 100)\(^{14}\).

**Abbreviations:** AC, acini; C, cercaria; CAC, compressed acinus; CT, connective tissue; DAC, disorganization of acinus; DG, digestive gland; LTP, larval trematode parasites (sporocysts and cercariae); NE, necrosis; O, ovary; OG, oogonia; PG, prostate gland; S, spermatozoa; SG, spermatogonia; SP, sporocysts; T, testis.

**In-vitro culture:** For the understanding of the physiology and biochemistry of larval digeneans, their in-vitro culture study is highly significant. Diverse techniques and culture media have been developed from time to time for culture of larval trematode parasites and work in this field has been nicely reviewed\(^{40}\). Commendable work has been carried out for in-vitro cultivation of metacercariae to their adults\(^{39,55}\).

In India, excysted echinostome metacercariae were successfully cultured in-vitro for seven days up to their vitellogenesis stage\(^{26}\). Indeed, in-vitro cultivation of larval digenean is also one of the good ways for the identification of various species of trematode parasites.

**Conclusion**

The present review on larval trematode parasites of freshwater snails reveals that majority of Indian workers have focused on cercariae spp. parasitizing freshwater snails inhabiting the lentic aquatic ecosystem. The most commonly reported cercariae are monostome, amhistome, echinostome, furcocercous, gymnocephalous and xiphidiocercous. Not much work has been done on lotic ecosystem dwelling snail species in the country, although a vast range of perennial rivers, canals, estuarine systems etc. are existing in India.

Studies on different aspects of biology of miracidium and metacercariae, the infecting stages are almost rare. Histopathologies and parasitic castration are well reported but studies at molecular level of parasitism are highly desired. At molecular level or genomic studies for the identification of various trematode larvae are also recommended in this era of science. The study on the regeneration of destructed organs caused by parasitism is also highly suggestive.

**References**


45. Karyakarte P.P. and Yadav B.B., Histochemical observations on the surface of amphistome larvae on the glycogen deposition in the hepatopancreas of *Indoplanorbis exustus*, *Nat. Sci. J.* , XV(8), 97-99 (1976)


68. Pandey K.C., On a rare cercaria, *Cercaria soparkarin* sp. (Transversotrematidae) from Lucknow, India, *J. Helm.*, XLV(4), 321-326 (1971)


76. Reader T.A.J., Histological and ultrastructural studies on the testis of *Bithynia tentaculata* (Mollusca: Gastropoda) and on the effects of *Cercaria helvetica* XII (Trematoda: Digenea) on this host organ, *J. Zoology*, 171(4), 541-561 (1973)


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