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ORIGINAL ARTICLE

First record of a rare transversotrematid cercaria larva (Trematoda: Digenea) from Rajasthan, India: focus on seasonal occurrence and host-specificity of diverse cercariae

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Abstract During the survey of freshwater snail hosts and their digenean larval trematode parasites, a rare cercaria larva belonging to family Transversotrematidae and subclass Digenea (Trematoda) was recovered from the snail species *Melanoides striatella tuberculata* inhabiting perennial Som river of Udaipur district, Rajasthan, India. More than 28 % mature specimens of these snails were found to be infected with transversotrematid cercaria larvae in the spring season. Body of this cercaria is large, bowl-shaped, biocellate, spinose, transparent and laterally extended having two pigmented eye spots, two hold fast organs extended from the junction of body and tail, large tail with two foliated furcal rami, and cyclocoel intestinal caeca. As far as the authors are aware, this is the first record of a transversotrematid larva from Rajasthan, India. Simultaneously, other forms of cercariae viz., amphistome, echinostome, monostome, gymnocephalous, furcocercous and xiphidiocercous cercariae were also recovered from fifteen species of pulmonate and operculate snails including *Lymnaea acuminata* f. *patula*, *L. acuminata* f. *chlamys*, *L. acuminata* f. *typica*, *L. acuminata* f. *rufescens*, *L. luteola* f. *australis*, *L. luteola* f. *typica, L. luteola* f. *impura*, *Planorbis* (*Indoplanorbis*) *exustus*, and *Anisus* (*Gyraulus*) *convexiusculus*, *Faunus ater*, *Melania* (*Plotia*) *scabra*, *Thiara* (*Tarebia*) *lineata*, *Melanoides striatella tuberculata*, *Vivipara bengalensis* race *gigantica* and *V. bengalensis* race *mandiensis*. The seasonal occurrence and host-specificity of diverse trematode cercaria larvae are also discussed besides the first record of a rare transversotrematid cercaria larva from Rajasthan, India.

Keywords Amphistome · Cercaria · Echinostome · Freshwater snail hosts · Furcocercous · Gymnocephalous · Host-specificity · Monostome · Seasonal occurrence · Transversotrematid larva · Xiphidiocercous · Rajasthan (India)

Introduction

It is well known that freshwater molluscs serve as intermediate hosts that facilitate completion of the life cycle of majority of digenetic trematode parasites. Indeed these molluscs harbor various developmental stages as sporocysts, rediae and cercariae of adult trematodes (Erasmus 1972). In India, these intra-molluscan larval stages have been well studied by several workers (Ganpati and Rao 1969; Singh 1959; Mukherjee 1966; Murty 1973, Mohandas 1974; Jain 1976; Pandey and Agarwal 1978; Choubisa and Sharma 1983a, Choubisa 1991a, 2010; Janardanan and Shiny 1989; Rajendran and Janardanan 1993; Sanil and Janardanan 2016) and different kinds of cercariae (amphistome, echinostome, furcocercous, gymnocephalous, monostome, xiphidiocercous etc.) and metacercariae (aspidogaster, echinostome, opisthorchid, plagiorchiid, strigeid etc.) from different geographical provinces have been reported.

In India, Rajasthan is the largest state where number of freshwater perennial lentic (ponds, reservoirs, dams etc.) and lotic (rivers. streams, canals etc.) habitats are found. Although, freshwater and terrestrial molluscan fauna have

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been studied and reported from almost every region of Rajasthan (Ray and Mukherjee 1963; Rathore and Bohra 1987; Choubisa 1992a; Choubisa and Sheikh 2013a, b, c). But studies on larval trematode parasites of freshwater snails are still too scanty (Choubisa 2008a). However, few excellent studies on nutrition and digestion (Sharma and Choubisa 1985; Choubisa 1988a, 2008b), neuroanatomy (Choubisa and Sharma 1983b; Choubisa 1986, 1988b, 1989), histopathogenesis (Choubisa1990a, 1998; Choubisa et al. 2012), parasitic castration (Choubisa and Sheikh 2013d), behaviors and seasonal infection (Choubisa 1991b, 1997, 2002; Choubisa and Sharma 1986) of diverse trematode larvae as well as on their in vitro culture (Choubisa 1988c) have been conducted earlier. Besides the new records of larval digeneans, few new species of cercariae have also been reported from Rajasthan (Sharma and Choubisa 1983; Choubisa 1985, 1990b, 1992b; Choubisa and Sharma 1985). Recently, from evolutionary point of view, a new morph of sporocyst larva has also been reported from Rajasthan (Choubisa and Sheikh 2013e). Nevertheless, works on freshwater snail fauna and their larval trematode parasites are still meager in Rajasthan; despite of its number of perennial freshwater habitats. Therefore, a survey was undertaken to ascertain the pulmonate and operculate snail host species inhabiting diverse lentic and lotic freshwater ecosystems of Rajasthan and their different kinds of cercaria larvae. Simultaneously, a focus was also made on their seasonal occurrence and host—specificity.

During the survey, in spring season, a rare transversotrematid cercaria larva was recovered from the bottom dwelling operculate snail hosts, *Melanoides striatella tuberculata* inhabiting the perennial Som river of Udaipur district of southern Rajasthan, India which is discussed in detail as a first record from Rajasthan.

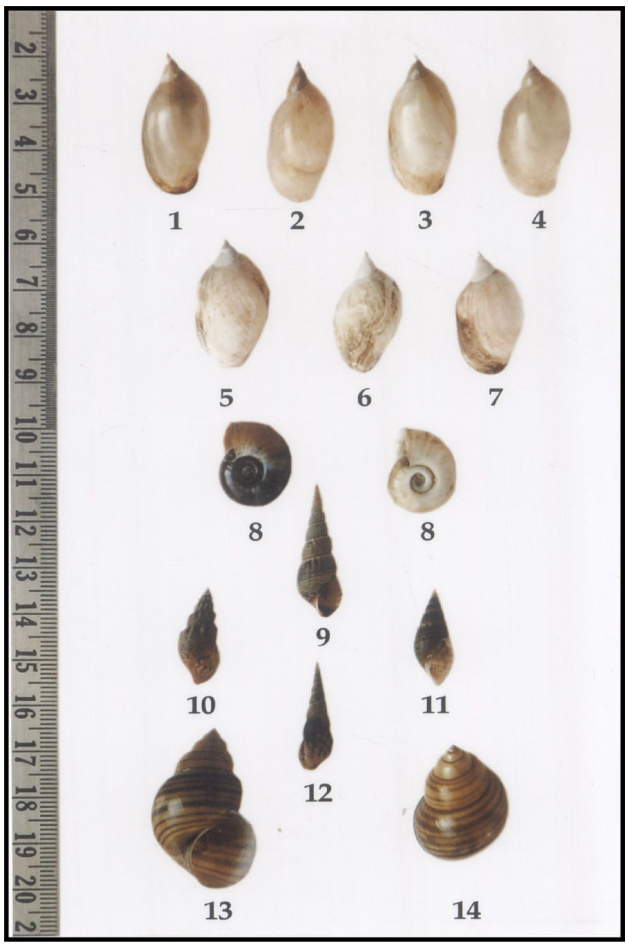

Fig. 1 Snail species collected from diverse freshwater lentic and lotic habitats of southern Rajasthan. (1) *Lymnaea acuminata* f. *patula* (2) *L. acuminata* f. *chlamys* (3) *L. acuminata* f. *typica* (4) *L. acuminata* f. *rufescens* (5) *L. luteola* f. *australis* (6) *L. luteola* f. *typica* (7) *L. luteola* f. *impura* (8) *Planorbis* (*Indoplanorbis*) *exustus* (9) *Faunus ater* (10) *Melania* (*Plotia*) *scabra* (11) *Thiara* (*Tarebia*) *lineata* (12) *Melanoides striatella tuberculata* (13) *Vivipara bengalensis* race *gigantica and* (14) *V. bengalensis* race *mandiensis*

Materials and methods

A survey was performed (2011–2012) for diverse forms of cercaria larvae infecting various snail species of freshwater perennial lentic (ponds, reservoirs, dams etc.) and lotic (rivers, streams, canals etc.) habitats of Rajasthan, India. For this purpose, mature living snail specimens 50 to 100 in numbers of *Lymnaea acuminata* f. *patula*, *L. acuminata* f. *chlamys*, *L. acuminata* f. *typica*, *L. acuminata* f. *rufescens*, *L. luteola* f. *australis*, *L. luteola* f. *typica, L. luteola* f. *impura*, *Planorbis* (*Indoplanorbis*) *exustus*, *Anisus* (*Gyraulus*) *convexiusculus*, *Faunus ater*, *Melania* (*Plotia*) *scabra*, *Thiara* (*Tarebia*) *lineata*, *Melanoides striatella tuberculata*, *Vivipara bengalensis* race *gigantica* and *V. bengalensis* race *mandiensis* (Fig. 1) were collected seasonally from almost each water body by hand or by hand net. These snails were then brought to the departmental laboratory and maintained in separate aquaria containing tap water and a few aquatic plants. Snails were also collected from aquatic weeds spread in water bodies. A record was also prepared for collected snails and their habitats. These snail species were identified morphologically or by their shell structure as described earlier (Tonapi 1980; Subba 1989).

Snail species of lentic or lotic habitat, of bottom-dwelling and surface-dwelling habits were maintained separately in laboratory aquaria. For examination, the snails were kept in 500 ml glass beaker containing clean tap water and then exposed to natural or artificial light for cercarial emergence and they were also dissected out for the presence of larval trematodes if needed. Cercaria larvae were collected with fine dropper and examined according

to the methods reported (Mukherjee 1980). The cercariae and other developmental stages were studied under light cover-slip pressure, both alive as well as in stained mounts. They were then identified following the standard key (Erasmus 1972).

Results and discussion

Transversotrematid cercaria larva

In survey of freshwater snail hosts and their digenean larval trematode parasites, a rare cercaria larva (Fig. 2a, b) belonging to family Transversotrematidae and subclass Digenea (Trematoda) was recovered from the snail host, *M. striatella tuberculata* (Fig. 1) belonging to family Melaniidae (Thiariidae) inhabiting perennial Som river of Udaipur district of Rajasthan, India (Fig. 3). More than 28 % of these snail species were found to be infected with transversotrematid cercarial larvae in the spring season. Interestingly, another snail species, *Melania* (*Plotia*) *scabra* (Fig. 1) was also recovered from the same habitat (Som river) which also belongs to the same family of snail *M. striatella tuberculata* but none of them was found to be infected with transversotrematid cercariae. It may be possible due to the difference in their niche. Although, emission of transversotrematid cercarial larvae from snail species, *M. scabra and M. striatella tuberculata* has been reported earlier from India (Nadakal et al. 1969), but this is the first report from Rajasthan.

The present transversotrematid cercariae were found strongly phototrophic, negatively geotactic and active swimmer. Although, they were diurnal but their maximum emergence was found in morning hours. Hence, these larvae are well adapted morphologically as they had two photosensitive pigmented eyes and tail bifurcated at the end region.

Moreover, transversotrematid cercariae exhibited large bowl-shaped, biocellate, spinose, transparent and laterally extended body, two hold fast organs/appendages extending from the junction of body and large tail with two foliated/spatulated furcal rami, and cyclocoel intestinal caeca. Other details of cercaria have been shown Fig. 2b. We neither took any body measurements nor counted the flame cells for flame cell formula. These tactics are generally used in identification of cercarial species besides the morphological features. However, in the present scenario, *study of trematode larvae at genetic or DNA level is probably an authentic way or technique for the confirmation as well as identification of cercarial species due to the innumerous variety of cercariae*. Molecular approaches for sensitive and specific detection of the parasite species in the snail host is not being widely used in India. Various PCR assays have been developed to detect DNA in fecal matter, definitive hosts and intermediate hosts outside India (Kozak and Wedrychowicz 2010; Caron et al. 2011). *Such techniques will also check the chances of duplication of cercarial species or fake work on taxonomy of trematode parasites and provide a key for future studies.*

In India, transversotrematid cercarial larva (*Cercaria patialensis*) was identified and reported for the first time from Punjab state (Soparkar 1924). Subsequently, Anantaraman (1948) from Nellore, south India, Nadakal et al. (1969) from Trivandrum, Kerala and Pandey (1971) from Lucknow, Uttar Pradesh have also reported it. From Rajasthan, this cercaria was detected for the first time. The presence of this trematode cercaria indicates that final hosts that are mostly fishes (Worawit and Kazuya 2012) in Som river are infected with adult transversotrematid parasites.

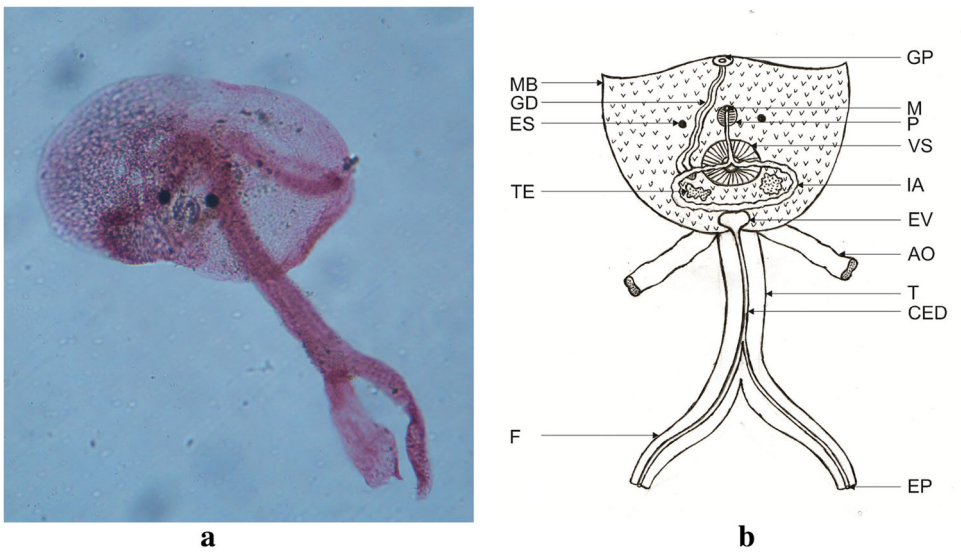

Fig. 2 A rare transversotrematid cercaria larva stained with Gower's carmine stain (Fig. 2a; 150x) and its diagrammatic sketch showing different organ systems as seen in alive cercaria (Fig. 2b). AO, adhesive organ; CED, caudal excretory duct, EP, excretory pore; ES, eye spot; EV, excretory vesicle; F, furca; GD, genital ducts; GP, genital pore; IA, intestinal arch; M, mouth; MB, main body; P, pharynx; T, tail; TE, testis; VS, ventral sucker

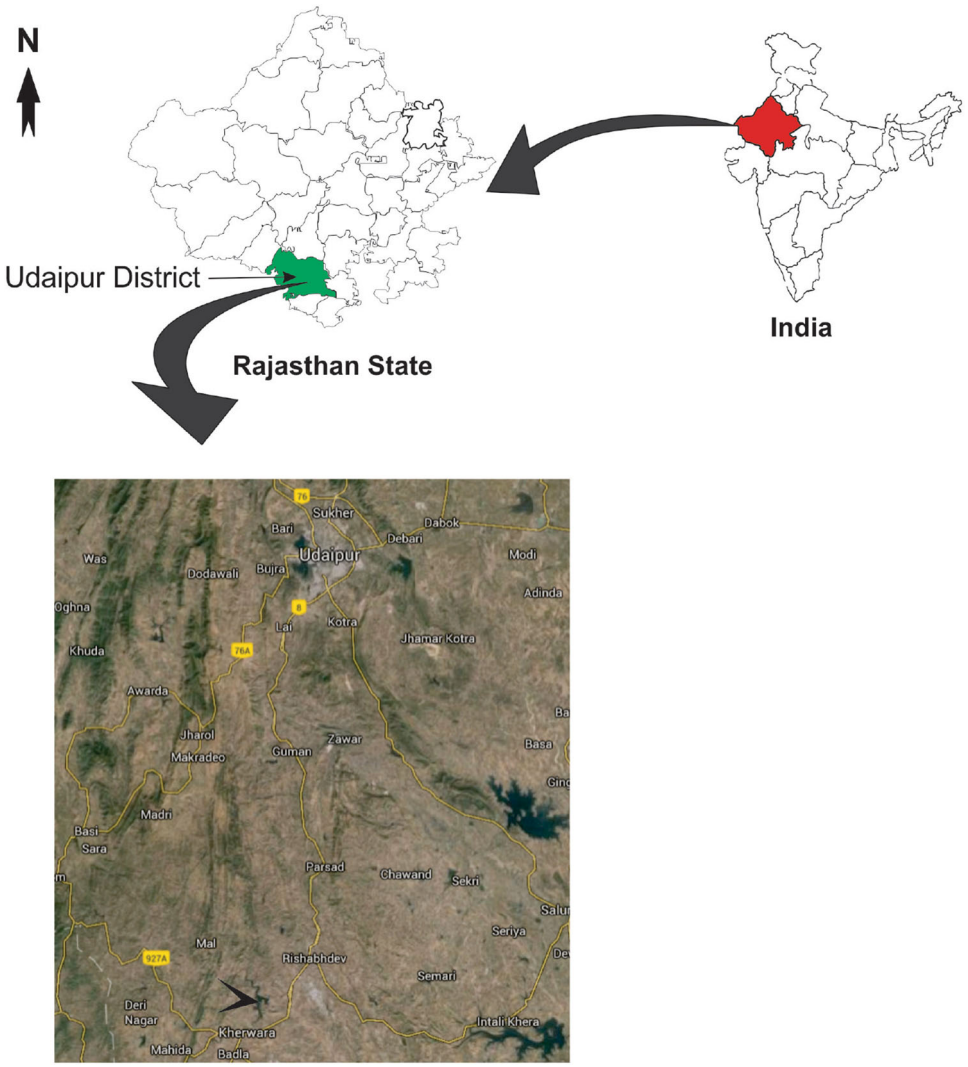

Fig. 3 Location of Som river (marked with arrow) flowing between Rishabhdev and Kherwara villages of Udaipur district of Rajasthan (India) from which snail hosts (*M. striatella tuberculata*) infected with transversotrematid cercariae were collected

Seasonal occurrence and behavior of diverse cercariae

In the present survey study a total of seven types or forms of cercariae viz., amphistomes, echinostomes, furcocercous, gymnocephalous, monostomes, transversotrematid, and xiphidiocercous were recovered from fifteen species of snail hosts viz., *L. acuminata* f. *patula*, *L. acuminata* f. *chlamys*, *L. acuminata* f. *typica*, *L. acuminata* f. *rufescens*, *L. luteola* f. *australis*, *L. luteola* f. *typica, L. luteola* f. *impura*, *P.* (*Indoplanorbis*) *exustus*, *A.* (*Gyraulus*) *convexiusculus*, *F. ater*, *M.* (*Plotia*) *scabra*, *T.* (*Tarebia*) *lineata*, *M. striatella tuberculata*, *V. bengalensis* race *gigantica* and *V. bengalensis* race *mandiensis* (Table 1).

Furcocercous, monostomes and transversotrematid cercariae were found to be relatively more active swimmers and exhibited strongly negative geotactic behavior and aggregated towards light source (highly phototactic).These larvae were not found to encyst on wall of container. Although, amphistome, echinostome and gymnocephalous cercariae were also phototropic but these were passive swimmers and got encysted within 5–10 min after emergence. Those cercariae bearing eye–spots were found to be strongly phototropic as compared to their counter parts. Such behaviors have also been observed by many workers (Erasmus 1972; Pandey and Agrawal 1977, 1978; Choubisa and Sharma 1986). The seasonal emergence or occurrences of different forms of cercariae from their snail-hosts have been depicted in Table 2. All kinds of cercariae were perfectly adapted morphologically to behaviors which help in successful completion of life cycle of digenetic trematode parasites.

Moreover, usually a single snail is infected with one type of larval trematode parasites at a time; however, in some cases the snails shed two types of cercariae exhibiting double infection. Snail species, *V. bengalensis, L. accuminata, M. striatella tuberculata* and *P. exustus*, revealed double infection. Not a single case of triple

Table 1 Snail species collected from diverse freshwater lentic and lotic habitats infected with different kinds of carcariae showing host-specificity as well

Snail species (gastropods)	Habitats (lo/le)	Cercariae						
		A	E	F	G	M	T	X
Pulmonates								
Family- Lymnaeidae								
1. *Lymnaea acuminata f. patula*	Le	−	+	+	+	−	−	−
2. *L. acuminata f. chlamys*	Le	−	+	+	+	−	−	−
3. *L. acuminata f. typical*	Le	−	+	+	+	−	−	−
4. *L. acuminata f. rufescens*	Le	−	+	+	+	−	−	−
5. *L. luteola f. australis*	Lo	−	−	+	+	−	−	+
6. *L. luteola f. typical*	Lo	−	−	+	+	−	−	+
7. *L. luteola f. impure*	Lo	−	−	+	+	−	−	+
Family- Planorbidae								
8. *Planorbis (Indoplanorbis) exustus*	Le + Lo	+	+	+	+	−	−	+
9. *Anisus (Gyraulus) convexiusculus*	Le	+	+	+	+	−	−	+
Operculates								
Family- Melaniidae (Thiaridae)								
10. *Faunus ater*	Le	−	−	+	−	+	−	−
11. *Melania (Plotia) scabra*	Lo	−	−	+	−	+	−	−
12. *Melanoides striatella tuberculata*	Le + Lo	−	−	+	−	+	+	+
13. *Thiara (Tarebia) lineata*	Le	−	−	+	−	+	−	−
Family-Viviparidae								
14. *Vivipara bengalensis race gigantica*	Le + Lo	−	−	+	−	−	−	+
15. *V. bengalensis race mandiensis*	Le + Lo	−	−	+	−	−	−	+

+, infected; −, uninfected

Le, lentic or stagnant waters (lakes, reservoirs, ponds, dams etc.); lo, lotic or running waters (rivers, streams, canals etc.)

A amphistome, *E* echinostome, *F* furcocercous, *G* gymnocephalous, *M* monostome, *T* transversotrematid, *X* xiphidiocercous cercariae

Table 2 Seasonal occurrence of cercarial infection in snail hosts

Types of cercaria	Seasonal occurrence/infection			
	Rainy (Jul- Sep)	Winter (Oct- Jan)	Spring (Feb- Mar)	Summer (Apr- Jun)
1. Amphistome	+++	++	+	+
2. Echinostome	+++	+++	+	+
3. Furcocercous	+++	++	+	++
4. Gymnocephalous	++	+++	++	+
5. Monostome	+++	++	++	+++
6. Transversotrematid	+	++	+++	+
7. Xiphidiocercous	+++	++	+	+

+, minimum; ++, moderate; +++, maximum

infection ever came across. Such findings have also been observed and reported by many investigators (Mukherjee 1966; Jain 1970; Pandey and Agrawal 1978; Choubisa 1984).

Host-specificity of cercarial larvae

It is interesting to note that monostome and transversotrematid species are restricted to snail host, *M. striatella*

tuberculata whereas amphistome, echinostome and gymnocephalous cercariae have been found to infect *P. exustus* and *A. convexiusculus, L. acuminata* and genera of Lymnaeidae family, respectively. Other cercariae, furcocercous and xiphidiocercous have been found to infect a number of snail species (Table 1). It appears that host-specificity and non-specificity is related to selection mechanism exhibited by miracidium, feeding behavior of the final host and part of the ecology of the intermediate hosts. Common genes of different molluscan host genera of a family are responsible for the infection with same larval forms whereas the difference in the host genes among the genera of the same family appear to be responsible for the difference in larval population. This view lends support from the work on gene based parasitic infection (Kennedy 1976) and from the definition given to parasitism by Mac Innis (1974) that *parasitism is defined as the case in which one partner, the parasite of a pair of interacting species is dependent upon a minimum of one gene or its product from the other interacting species defined as the host for survival*.

In conclusion, to the best of our knowledge, a rare transversotrematid cercarial recovered from *M. striatella tuberculata* is the first record from Rajasthan (India).The findings of present study are highly significant and add to existing knowledge of modern parasitology. Authors suggest that cercariae should be identified by their genetic study instead of using of flame cell formula.

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