New species of macrocercous and megalurus cercariae infecting the freshwater snail, *Paludomus (Paludomus) tanschauricus* (Gmelin, 1771) in Malabar, Kerala

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Abstract The present paper describes two new species of cercariae infecting the freshwater snail, *Paludomus (Paludomus) tanschauricus* from Malabar, Kerala. *Paludomus tanschauricus* collected from the hill streams at Vattakkundu in the Wayanad district of Kerala harboured a macrocercous cercaria, *Cercaria* sp. XX Malabar n. sp. characterized by a disproportionately large tail. Developmental stages were recovered from the hepatopancreatic tissues of the snail host. Cercariae encysted on the gills of the fish, *Haludaria melanampyx*. Snails collected from Papanashini rivulet at Thirunelli, also from Waynad district were infected with a megalurous cercaria, *Cercaria* sp. XXI Malabar n. sp. possessing an elongated tail with an adhesive cup-like sucker at its tip. Redial stages developed in the hepatopancreas while the cercariae were found to encyst on any available substrate. The morphology and morphometry of these two cercariae along with their developmental stages are studied in detail and compared with related species to establish their systematic positions.

Keywords Macrocercous · Megalurous · Cercaria · Sporocyst · *Paludomus (Paludomus) tanschauricus* · Malabar · Kerala

Introduction

The freshwater snails of the genus *Paludomus* (Swainson, 1840) inhabiting the hilly streams and rivulets are widely distributed across the peninsular India, Myanmar and Sri Lanka (Subba Rao 1989; Ramakrishna and Dey 2007). However, very little information is available on the cercariae infecting snails of this genus from India. Sewell (1922) described five species of cercariae (*Cercariae indicae* XXXIII, *C. indicae* XXXVII, *C. indicae* XXXVIII, *C. indicae* XLV and *C. indicae* LVIII) from *Paludomus*. Peter and Srivastava (1955) described *Cercaria ratnagiriensis*, while Vasandakumar and Janardanan (2005, 2006) described *Cercaria* sp. III Malabar, *Cercaria* sp. IV Malabar and *Cercaria* sp. V Malabar from *Paludomus* spp. Of the nine species of cercariae described above, except *C. ratnagiriensis*, all eight species were reported from Kerala. An extensive study on the cercarial fauna of *P. (Paludomus) tanschauricus* inhabiting the hill streams in the Malabar region of Kerala revealed the presence of two species of cercariae. Comparison of characters of the present cercariae with related species indicated that they are distinctly different from other known species of macrocercous and megalurous cercariae and, are therefore reported here as new species.

Materials and methods

Paludomus (Paludomus) tanschauricus were collected from a hill stream at Vattakkundu and Papanashini rivulet at Thirunelli in Wayanad district of Kerala from July 1989 to October 1991. The snails were brought live and maintained in the laboratory. After screening for cercarial infections, the infected snails were isolated and the

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emerging cercariae were studied alive, with or without vital staining using a phase-contrast research microscope (Carl Zeiss, Germany). Genital primordia were studied after staining with lacto-acetic carmine. Rediae recovered from the digestive glands of infected snails were stained supravitaly with neutral red and studied in detail. Metacercarial cysts were isolated, ruptured using a fine needle and studied. Measurements were taken from 10% formalin-fixed specimens ($n = 30$), are expressed in micrometres (μm) as range, followed by mean in parentheses. Figures were drawn with the aid of a camera lucida and based on observations made on live specimens, details were added free-hand.

Results and discussion

Cercaria sp. XX Malabar n. sp.

Host: *Paludomus* (*Paludomus*) *tanschaureicus* (Gmelin, 1771).

Site of infection: Hepatopancreas

Type locality: Vattakkundu in Wayanad district, Kerala

Collected by: N. K. Sanil

Holotype: Deposited in the parasite collections of Parasitology Laboratory, Department of Zoology, University of Calicut (Accession number: CM.1.1).

Etymology: The name 'Malabar', refers to the region from where the species was recorded

Period of collection: July, 1989 to September, 1991

Prevalence: 0.31% (Two of 654 *P. tanschaureicus* screened were infected)

Cercarial behavior Prefers to emerge during daytime; exhibited strong, positive phototaxis and swam actively by wriggling their tails, resembling small worms/larvae.

Description (Fig. 1a)

Distome, macrocercous cercaria with an oval to elongate-oval, highly contractile, aspinose body measuring, $158.4\text{--}244.2$ (198.8) $\times$ $102.2\text{--}135.3$ (118.0) in size. Possesses a thick body wall, lateral body fields packed with refractile, cystogenous material, obscuring internal structures; six pairs of sensory hairs present on body. Tail terminal; disproportionately large, many times the body in size; annulated when contracted, $544.5\text{--}1158.3$ (828.6) $\times$ $72.6\text{--}108.9$ (94.6) in size; without spines and sensory hairs. Eight to 12 small, slit-like openings present on the ventral side of tail, but were not connected with caudal excretory canal. Just behind the oral sucker, a prominent prepharyngeal body measuring 13.2×9.9 present. Oral sucker terminal, almost circular, measured

Fig. 1 *Cercaria* sp. XX Malabar n. sp. **a** cercaria, **b** redia, **c** metacercaria

$33.0\text{--}39.6$ (36.9) $\times$ $33.0\text{--}39.6$ (36.6); a few bud-like structures, resembling duct openings seen at the anterior margin of oral sucker. A post-equatorial, muscular, ventral sucker measuring $36.3\text{--}42.9$ (39.3) $\times$ $36.3\text{--}42.9$ (37.8) in size present. A circlet of small, 29–32, inwardly directed, bud-like structures present on ventral sucker.

Mouth terminal; followed by a long prepharynx and pear-shaped, muscular pharynx which measured $19.8\text{--}26.4$ (22.8) $\times$ $13.2\text{--}16.8$ (13.7). Oesophagus long, narrow, bifurcates anterior to ventral sucker; caecal tips not discernible due to heavy deposition of cystogenous material in the posterolateral fields of the body. Small gland-like cells observed posterolateral to oral sucker, but gland ducts could not be traced. Genital primordium located anterior to ventral sucker, composed of three distinct lobes, one anterior and two posterior, interconnected by groups of cells.

A transversely elongate, thin-walled, saccular, excretory bladder located at the posterior end of the cercarial body. Anterior region of the bladder gives rise to a pair of primary collecting vessels. Each primary vessel runs forward, forms a loop just below the oral sucker, takes a 'U' turn and anterolateral to oesophagus, divides into anterior and

posterior collecting vessels. Five ciliary patches, two in anterior and three in posterior collecting vessels. Posteriorly, the excretory bladder extends into the tail as a trilobed structure, separated by a constriction at the body-tail junction and continues behind as caudal excretory canal; external opening not discernible. Flame cell formula: $2[(2 + 2) + (2+2)] = 16$.

Redia: (Fig. 1b)

Rediae develop in the digestive gland of infected *P. tanschauricus*. Body brownish with prominent collars and locomotor organs and measured 488.0–1215.0 (912.2) $\times$ 162.0–195.0 (181.0) in size. Pharynx well developed, muscular; gut with yellowish contents extends half to three-fourth the redial length. Redia enclosed numerous immature cercariae and germ balls; mature cercaria were observed free in liver matrix.

Metacercaria: (Fig. 1c)

Natural infections with metacercariae were observed in the gills of the fish, *Haludaria melanampyx* (Day) collected from the same habitat. Metacercariae attached to gill rakers at the basal region of gill filaments, measured 116.1–127.7 (123.0) $\times$ 81.0–93.3 (88.0). Experimentally infected metacercariae develop, attached to gill filaments in *H. melanampyx*; were oval in shape with a single layered cyst wall.

Remarks

Comparison of the present cercariae with previously described macrocercous cercariae reveals that it shows affinities to *Cercaria pyrgophspiralis* Nasir and Diaz, 1973, cercaria of *Mesorchis denticulatus* Koi, 1986, cercaria of *Echinochasmus macrocaudatus* Ditrich, Scholz and Vargas-Vazquez, 1997, cercaria of *Stephanoprora uruguayense* Ostrowski de Núñez, 2007 and cercaria of *Stephanoprora aylacostoma* Ostrowski de Núñez and Quintana, 2008.

Characters and measurements of the present cercaria are compared with that of the above five forms in Table 1. *C. pyrgophspiralis* deviates from the present cercaria in having a spinose body, eight spine-like structures on oral sucker and refractile material in main excretory tubules. Also, in *C. pyrgophspiralis*, secondary excretory tubules bifurcate lateral to oesophagus, whereas in present form it is anterolateral. Cercaria of *M. denticulatus* varies from the present form in measurements of the tail, spination of body, in having a collar with 22 spines, spines on oral and ventral suckers and in the absence of prepharyngeal body. In the measurements of body, tail, suckers and pharynx, and in

the absence of prepharyngeal body the present form stands apart from cercaria of *E. macrocaudatus*. While, variations in measurements of body, suckers and pharynx and possession of a head collar and short prepharynx separates cercaria of *S. uruguayense*. Cercaria of *S. aylacostoma* stands apart from the present cercaria in measurements of body, tail and ventral sucker. Further, the present cercaria differs markedly from all the compared forms in the organization of caudal excretory canal and in its snail host.

In view of these differences, the present cercaria needs to be considered as a new species and is therefore, reported here as *Cercaria* sp. XX Malabar n. sp.

Cercaria sp. XXI Malabar n. sp.

Host: *Paludomus* (*Paludomus*) *tanschauricus* (Gmelin, 1771)

Site of infection: Hepatopancreas

Type locality: Papanashini rivulet at Thirunelli in Wayanad district, Kerala

Collected by: N. K. Sanil

Holotype: Deposited in the parasite collections of Parasitology Laboratory, Department of Zoology, University of Calicut (Accession number: CM.2.1).

Etymology: The name ‘Malabar’, refers to the region from where the species was recorded

Period of collection: October, 1991

Prevalence: 0.31% (Two of 654 snails screened were infected)

Cercarial behavior Cercarial emergence was observed throughout the day and night; change of water stimulates emission. Are active swimmers, propelling itself by the lashing, whip-like movements of their tails and were occasionally attached to water surface. Prefer to attach to moving objects, followed by encystment; also attach and encyst on walls of container with light serving as strong stimulus for encystment. Cercariae swim actively for prolonged periods in darkness.

Description: (Fig. 2a, b)

Belongs to the Megalurous group of cercaria. Cercarial body oval to elongate in shape, highly contractile with a slight constriction at the level of ventral sucker; measured 431.2–646.8 (507.4) in length and 215.6–292.6 (247.9) in width. Body aspinose with a few sensory hairs on the surface. Except the anterior third, body covered with rod-like, refractile, cystogenous material. Possesses a terminally placed, long, highly contractile and aspinose tail, measuring 585.2–1216.6 (871.6) $\times$ 69.3–84.7 (79.3). Tail tip modified into a cup-like sucker with a central lumen surrounded by four columnar, gland-like cells, each with

Table 1 Showing comparative characters and measurements of *Cercaria* sp. XX Malabar n. sp. and five related cercariae

Cercaria (hosts)	<i>Cercaria pygophosphoralis</i> Nasir and Diaz, 1973 (<i>Pygophorus spiralis</i>)	Cercaria of <i>Mesorchis denticulatus</i> Koie, 1986 (<i>Hydrobia ulvae</i>)	Cercaria of <i>Echinochasmus macrocaudatus</i> Ditrich, Scholz & Vargas-Vazquez, 1996 (<i>Pygophorus coronatus</i>)	Cercaria of <i>Stephanoprora uruguayense</i> Ostrowski de Núñez, 2007 (<i>Heleobia parchappei</i>)	Cercaria of <i>Stephanoprora aylacostoma</i> Ostrowski de Núñez and Quintana, 2008 (<i>Aylacostoma chloroticum</i>)	<i>Cercaria</i> sp. XX Malabar n. sp. (<i>Paludomus (Paludomus) tanschauricus</i>)
Body	Spinose, 150–165 × 60–76	Spinose, 190–250 (210) × 100–130 (110)	Aspinose, oval, 242–258 × 203–221.	Aspinose, 170–198 (183) × 57–79 (71)	Aspinose, 239–264 (250) × 82–94 (88)	Aspinose, 158.4–244.2 (198.8) × 102.2–135.3 (117)
Tail	Aspinose, 517–582 × 37–56	Aspinose, 564–2000 × 72–150	Aspinose, 722–987 × 153–263	Aspinose, 800–1088 (991) × 64–112 (96)	Aspinose, 1792–2224 (1919) × 138–192 (161)	Aspinose, 544.5–1158.3 (828.6) × 72.6–108.9 (94.6)
Oral sucker	25–30	40–46 (42)	54–56 × 54–55	28–32 (31) × 28–35 (32)	35–47 (39) × 38–44 (40)	33–39.6 (37) × 33–39.6 (36.7)
Ventral sucker	25–30	40–45 (42)	50–52 × 50–52	32–38 (35) × 32–38 (34)	41–47 (46) × 38–44 (42)	36.3–42.9 (39.3) × 36.3–42.9 (37.8)
Prepharyngeal body	–	–	–	13, with 2 globular inclusions	Small	Small, 13.2 × 9.9
Prepharynx	Long, 15–24	Long, 18–22 (20)	32–37	Short	25–32 (28)	Long
Pharynx	15–21 × 10–12	Oval to pyriform 18–22 (20) × 16–20 (18)	30–33 × 18–22	Muscular, 13–19 (16) × 13–16 (14)	Muscular, 16–25 (20) × 13–16 (14)	Muscular, 19.8–26.4 (22.8) × 13.2–16.8 (13.7)
Oesophagus	Well developed	Long, narrow	32–45	Long	Long	Long, narrow
Caeca	Broad, extend to posterior end	Long, extend to posterior level of VS	Terminating at the middle of acetabulum	Long, reaching the level of excretory vesicle	Long, reaching the level of excretory vesicle	Caecal tips not clear
Excretory bladder	Lobose	Spherical to oval	Saccular, constricted at body–tail junction	Saccular	Saccular	Saccular, constricted at body–tail junction
Caudal excretory canal	–	Bifurcates in the posterior third	Open out in the anterior third of tail	Bifurcates unequally	Ends blindly in the anterior portion of tail	With a tri-lobed structure at the anterior end of tail
Flame cell formula	2 [(2 + 2 + 2 + 2)] = 16	2 [(2 + 2) + (2 + 2)] = 16	2 [(2 + 2) + (2 + 2)] = 16	2 [(2 + 2) + (2 + 2)] = 16	16 pairs	2 [(2 + 2) + (2 + 2)] = 16

Fig. 2 *Cercaria* sp. XXI Malabar n. sp. **a** cercaria, **b** genital primordium, **c** redia, **d** metacercaria

clear nuclei and granular contents; lumen communicating to outside by the open end of the cup. Numerous parenchymatous cells and caudal bodies observed in tail stem. Caudal excretory canal short, bifurcates a short distance from tail base and opens out laterally. Oral sucker slightly longer than wide and measured 77.0–92.4 (81.6) in length and 69.3–77.0 (74.7) in width. Post-equatorial ventral sucker, muscular, wider than long and measured 77.0–100.1 (87.8) $\times$ 84.7–107.8 (98.6). Subterminal mouth leads to a short prepharynx followed by a muscular pharynx. Pharynx wider than long and measured 30.8–46.2 (38.5) $\times$ 33.5–46.2 (40.8). Oesophagus short; caeca long with caecal tips extending up to the posterior end of body. Anterolateral to ventral sucker, six pairs of penetration glands present. Gland ducts run forward and open out independently, anterior to oral sucker. Genital primordium represented by two distinct, interconnected masses of cells, overlapping the ventral sucker. Excretory bladder saccular, thin-walled, occupied the posterior end of cercarial body. A pair of primary collecting vessels emanate from the lateral wall of the excretory bladder. Each primary vessel run forward and adjacent to pharynx, coil upon itself and runs down and anterolateral to ventral sucker, bifurcates into anterior and posterior collecting vessels. Flame cell

formula: 2 [(3 + 3 + 3 + 3 + 3) + (3 + 3 + 3 + 3 + 3)] = 60.

Redia: (Fig. 2c)

Developing in the digestive tissues of the snail host, redia measured 688–1496 (1049.0) $\times$ 280–392 (326.5); possesses a muscular pharynx and a gut which extended half to three-fourth the redial length with greenish/ brownish contents. Redia contained a few mature and immature cercariae and very few germ balls.

Metacercaria: (Fig. 2d)

Attachment and encystment of cercaria takes place in presence of light and the process is accelerated by movement/mechanical stimuli. Moving bodies/objects are always preferred for attachment. However, in the absence of such stimuli, it can attach to any substratum including walls of the container. Encystment process begins with the attachment of tail to the substratum with the help of the adhesive sucker, followed by the attachment of cercarial body. A cyst wall is formed around the cercarial body and subsequently the tail gets detached. Cyst measured 237.2–291.0 (253.8) $\times$ 218.6–240.4 (231.0) in size; cyst wall composed of three layers, an innermost thin and translucent layer, a middle thicker layer and an outermost thickest layer.

Remarks

When compared with megalurous cercariae possessing an elongate tail with an adhesive organ at its tip, the present cercaria closely resembles *Cercariae indicae* IV Sewell, 1922, *Cercaria caudiglandula* Premvati, 1954, *Cercaria ratnagiriensis* Peter and Srivastava, 1955, cercaria of *Cloacitrema philippinum* Velasquez, 1969 and *Cercaria melanopsi* VIII Ismail and Hafez, 1984.

However, the present cercaria stands out from the above five forms in many aspects; a comparison of characters and measurements is made in Table 2. It varies from all the above forms except cercaria of *C. philippinum* in having an aspinose body, from all cercariae except *C. indicae* IV in the number of caudal gland cells and from *C. indicae* IV, *C. caudiglandula*, *C. ratnagiriensis* and cercaria of *C. philippinum* in the number of penetration glands. It again differs from *C. indicae* IV and *C. ratnagiriensis* in the position/arrangement of penetration glands. Further, it can be differentiated from all the above compared forms in the organization of excretory system, flame cell formula and in its snail host.

Cercaria (host)	<i>Cercariae indicae</i> IV Sewell, 1922 (<i>Melanoides lineatus</i> , <i>M.</i> <i>tuberculatus</i> , <i>M. scabra</i>)	<i>Cercaria caudiglandula</i> Premvati, 1954 (<i>Melanoides</i> <i>tuberculatus</i>)	<i>Cercaria ratnagiriensis</i> Peter and Srivastava, 1955 (<i>Paludomus obesa</i>)	<i>Cercaria of Cloacitrema</i> <i>philippinum</i> Velasquez, 1969 (<i>Cerethium ornata</i>)	<i>Cercaria melanopsi</i> VIII Ismail and Hafez, 1984 (<i>Melanopsis praemorsa</i>)	<i>Cercaria</i> sp. XXI Malabar n. sp. (<i>Paludomus (Paludomus)</i> <i>tanschaureicus</i>)
Body	Spinose, 421–789 × 105–210	Spinose, 750 × 270	Spinose, 490–980 × 70–250	Spinose, 365–650 × 115–165	Spinose, 450–650 × 70–130	Spinose, 431–646 (507) × 215–292 (247)
Tail	Spinose, 386–614 × 35–44	Spinose, 530 × 90	Spinose, 320–1510 × 20–90	Spinose	Spinose, 625–800 × 33–43	Spinose, 585.2–1216.6 (871.6) × 69.3–84.7 (79.3)
Caudal gland cells	4	12–14	8	–	6	4
Oral sucker	61	100	107	50–60 × 50–65	70 × 85	77.0–92.4 (81.6) × 69.3–77.0 (74.7)
Ventral sucker	68–70	100	128	60–85 × 60–70	95–100	77–100 (87) × 84–107 (98)
Penetration glands	10–12 pairs	9 pairs	8 pairs	3 pairs	6 pairs	6 pairs
Prepharynx	Short	Elongate, narrow	Present	Long, 20–50	Long	Short
Pharynx	Muscular, 25 × 18	Muscular	Pear-shaped, 75 × 65	20–40 × 20–35	Muscular, 42 × 30	Muscular, 30–46 (38) × 33–46 (40)
Oesophagus	Moderately wide	Broad, short	Short, broad	Short	Short	Short
Caeca	Till posterior end	Till posterior end	Till excretory bladder	Till posterior end	Till posterior end	Till posterior end
Excretory bladder	Pyriform	Bulbous	Pyriform	Elongated	Globose	Saccular
Caudal excretory canal	Absent	Not seen	Absent	–	Not seen	Bifurcate a short distance from tail origin
Flame cell formula	At least 14 pairs	13 pairs	12 pairs	–	2 [(2 + 2 + 2 + 2 + 2 + 2 + 2 + 3 + 3 + 3 + 3) + 3 + 2 + 1 + (2)] = 40	2 [(3 + 3 + 3 + 3 + 3 + 3) + 3 + 3 + 3 + 3 + 3] = 60

Considering the above differences, the present cercaria appears to be a new species and is, therefore, reported here as *Cercaria* sp. XXI Malabar n. sp.

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Author's contribution Dr. KPJ designed and guided the study. Dr. NKS carried out the survey, collected and studied the cercariae in detail. The manuscript was written by both the authors.

Compliance with ethical standards All the required ethical standards were complied with.

Conflict of interest The authors declare that there is no conflict of interest between them.

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