Two new species of furcocercous cercariae infecting the fresh water snail, *Thiara tuberculata* (Müller) in Kozhikode and Malappuram districts of Kerala

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Abstract Two new species of furcocercous cercariae, *Cercaria* sp. XVI Malabar n. sp. and *Cercaria* sp. XVII Malabar n. sp. were recovered from the freshwater snail, *Thiara tuberculata* in the Malabar region of Kerala. *Cercaria* sp. XVI Malabar n. sp., a pharyngeate, longifurcate furcocercous cercaria with two pairs of penetration glands and 10 pairs of flame cells was recovered from *T. tuberculata* collected from Kundayithode in Kozhikode district and Nilambur in Malappuram district of Kerala. *Cercaria* sp. XVII Malabar n. sp., another pharyngeate, longifurcate cercaria with a rudimentary ventral sucker was recovered from the same snail host collected from Nilambur in Malappuram district. Sporocysts of *Cercaria* sp. XVI Malabar n. sp. developed in the hepatopancreas, while that of *Cercaria* sp. XVII Malabar n. sp. were found developing in both hepatopancreas and stomach wall. The present paper describes the new species of cercariae in detail and compares them with related species to establish their systematic position.

Keywords Furcocercous · *Cercaria* · Sporocyst · *Thiara tuberculata* · Malabar · Kerala

Introduction

Information on furcocercous cercariae infecting the freshwater snail, *Thiara tuberculata* (Müller) in the Malabar region of Kerala is limited to that of two species, *C. indicae* XLVII (Sewell 1922), and *Cercaria* sp. 1 Malabar (Vasandakumar and Janardanan 2005). An extensive survey of *T. tuberculata* inhabiting freshwater bodies in Malabar revealed the presence of two species of furcocercous cercaria. These are morphologically and morphometrically distinct from all previously described furcocercous cercariae and are therefore reported here as new species. The cercariae are designated by Roman numerals, followed by Malabar to indicate the region of collection.

Materials and methods

Fresh water snails were collected from paddy fields, ponds, streams and canals in various localities of Malabar, from April 1988 to August 1991. Collected snails were transported live and maintained in the laboratory. Infected snails were identified, isolated and kept individually in beakers containing seasoned tap-water. Freshly emerging cercariae were studied live, with or without vital staining, using phase-contrast optics of a Carl Zeiss phase-contrast research microscope. After completing the cercarial study, the infected snails were crushed and examined for intra-molluscan stages of development. Sporocysts were studied after supra-vital staining with neutral red. Measurements were taken from specimens ($n = 30$) fixed in 10% formalin and are given in micrometres (μm) as range followed by mean in parentheses. Figures were drawn with the aid of a camera lucida and details from live observations were added free-hand.

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Results and discussion

Cercaria sp. XVI Malabar n. sp

Host: *Thiara tuberculata* (Müller)

Site of infection: Digestive gland

Type locality: Kundayithode in Kozhikode district and Nilambur in Malappuram district, Kerala

Collected by: N. K. Sanil

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

Etymology: The name refers to the region (Malabar) from which the species was recorded for the first time
Period of collection: November, 1989–August 1990; December, 1990

Prevalence: Eighteen of 665 snails (2.71%) screened were infected

Cercarial emergence starts during morning hours. Cercariae are active swimmers; swimming interrupted by brief periods of rest, during which, the furcal rami are spread out with body hanging down at an angle to tail stem.

Description (Fig. 1a, b)

Distome, pharyngeate, longifurcate furcocercous cercaria. Body elongate, measured 158.4–210.3 (179.7) × 49.5–69.3 (57.8), spinose; anteriorly a group of six to eight large spines seen overhanging the mouth; spines in the region of anterior organ large, heavily distributed, followed by a region of small spines near pharynx; remaining body surface armed with lightly distributed, large spines. Five pairs of sensory hairs present on body. Tail terminal, larger than body, aspinose; measured 184.8–217.8 (198.2) × 29.7–42.9 (38.6); numerous sensory hairs present on anterior and posterior quarters of tail. Furca longer than tail stem, 201.3–237.6 (221.0) × 19.8–26.4 (23.6) in size, not constricted off, aspinose, finfolds absent; two pairs of sensory hairs present on each furca. Six to eight pairs of large, parenchymatous cells present in tail stem, caudal excretory canal narrow, bifurcates and open out at the mid-region of each furcal rami. Anterior organ terminal, pear-shaped, measured 39.6–52.6 (44.8) × 23.1–26.4 (24.9). Ventral sucker post-equatorial, 19.8–23.1 (22.4); armed with a circlet of 30 large, inwardly directed spines. Mouth terminally located; prepharynx short, leading to a pear-shaped pharynx, measured 9.6–9.9 (9.8) × 6.6–9.9 (7.6); oesophagus narrow; caeca elongate, extending to the posterior region of body. Two pairs of penetration glands, located pre- and post-acetabular; glands irregular in outline, with conspicuous nuclei and finely granular contents; gland ducts empty lateral to mouth. Genital primordia consist of an irregular mass of cells behind

Fig. 1 *Cercaria* sp. XVI Malabar n. sp. **a** Cercaria, **b** sporocyst

ventral sucker. Excretory bladder transversely elongate, bicornuate; primary collecting vessels arise from antero-lateral margin of excretory bladder, take a convoluted course and lateral to ventral sucker, bifurcate into anterior and posterior collecting vessels. Flame cell formula: 2 [(2 + 2) + (2 + 2) + (2)] = 20.

Sporocyst (Fig. 1c)

Sporocysts develop in the digestive gland of infected *T. tuberculata*. Body filamentous, measured 2121.6–4165.2 (3143.4) × 171.6–374.4 (273.0); accommodated two or three cercariae and germ balls.

Remarks

Among the longifurcate, pharyngeate, furcocercous cercariae possessing two pairs of penetration glands and 10 pairs of flame cells, the present cercaria comes close to

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

Cercaria (Hosts)	<i>Cercaria pseudovaricata</i> Faust 1924 (<i>Semisulcospira libertina</i>)	<i>Cercaria spiralis</i> Probert 1966 (<i>Lymnaea peregra</i>)	<i>Cercaria leytensis</i> no. 2 Ito et al. 1977 (<i>Oncomelania quadrasi</i>)	<i>Cercaria shizuokaensis</i> Ito 1978 (<i>Austropeplea ollula</i>)	<i>Cercaria</i> sp. XVI Malabar n. sp. (<i>Thiara tuberculata</i>)
Body	Spinose, 135–153 (146) × 64–86 (74)	Spinose, 140–165 (154) × 40–70 (50)	Spinose, 106–128 (114) × 52–70 (62)	Spinose, 113–168 (140) × 55–62 (58)	Spinose, 158.4–210.3 (179.7) × 49.5–69.3 (57.8)
Tail	Spinose, 150–180 (163) × 37–42 (39)	Spinose, 174–185 (180) × 35–48 (40)	Spinose, 130–160 (148) × 30–40 (36)	Spinose, 100–144 (125) × 38–48 (40)	Spinose, 184.8–217.8 (198.2) × 29.7–42.9 (38.6)
Furcae	Spinose, finfold absent, 145–171 (151) × 13–18 (16)	Spinose, finfold absent, 170–190 (180) × 19–26 (22)	Spinose, finfold absent, 140–190 (165) × 15–22 (18)	Spinose, finfold absent, 149–161 (154) × 12–17 (15)	Spinose, finfold absent, 201.3–237.6 (221.0) × 19.8–26.4 (23.6)
Anterior organ	32–37 (34) × 32–47 (39)	38–48 (43) × 24–28 (26)	20–30 (26) × 19–28 (24)	29–36 (32) × 26–34 (29)	Pear-shaped, 39.6–52.6 (44.8) × 23.1–26.4 (24.9)
Ventral sucker	29–34 (29) × 32–34 (32)	26–32 (30) × 26–28 (28), with 3 rows of spines	17–22 (20) × 20–30 (24)	26–34 (29) × 29–36 (31), with 7 rows of spines	Post-equatorial, 19.8–23.1 (22.4), single row of spines
Prepharynx	Absent	Short	Absent	Short	Short
Pharynx	15–19 (16) × 16–21 (18)	Muscular	Present	Globular	Pear-shaped, 9.6–9.9 (9.8) × 6.6–9.9 (7.6)
Oesophagus	Short	Short	Short	Short	Long
Caeca	Long, extends up to excretory bladder	Long, septate, extends up to excretory bladder	Long, extends up to posterior end	Long, extends up to posterior end	Long, extends to the posterior quarter of body
Excretory bladder	Cup-shaped	Two chambered	Small, globular	Globular	Transversely elongate, bicornuate
Caudal excretory canal	Open at one-third from furcal origin	Open in posterior half of furcal rami	Open at posterior third of furca	Bifurcate into furca, opening not clear	Open out at one-third from furcal origin
Flamecell formula	2 [(2 + 2) + (2+2 + (2)))] = 20	2 [(4) + (4 + (2)))] = 20	2 [(2+2) + (2+2 + (2)))] = 20	2 [(2+2) + (2+2 + (2)))] = 20	2 [(2+2) + (2 + 2 + (2))] = 20

Cercaria pseudovaricata Faust 1924, *Cercaria spiralis* Probert 1966, *Cercaria leyteensis* no. 2 Ito et al. 1977 and *Cercaria shizuokaensis* Ito 1978.

Characters and measurements of the above four cercariae are compared with that of the present form in Table 1. *C. pseudovaricata* differs from the present form in shape and measurements of body, tail, anterior organ and ventral sucker; in having a spinose tail longer than furca and in the position of penetration glands. *C. spiralis* distinguishes itself from the present form in body shape, possessing short furca, large ventral sucker, septate caeca, three circlets of spines on ventral sucker, absence of sensory hairs on body, arrangement/position of penetration glands and in having swollen gland duct endings. *Cercaria leyteensis* no. 2 differs from the present cercaria in measurements of body and tail, in having a spherical penetration apparatus, spinose furca, in the position of caudal excretory canal opening and in the size, position/arrangement of penetration glands. *C. shizuokaensis* differs from the present form in having short tail and furca, spines on furca, eyespots and seven circlets of spines on ventral sucker; in the position of caudal excretory canal and in the position, shape and size of penetration glands. Further, the present cercaria develops in a different snail host. In the light of the differences with related forms, it is reasonable to treat the present cercaria as a new species and the name *Cercaria* sp. XVI Malabar n. sp. is proposed for it.

Cercaria sp. XVII Malabar n. sp

Host: *Thiara tuberculata* (Müller)

Site of infection: Digestive gland and wall of stomach

Type locality: Nilambur in Malappuram district, Kerala

Collected by: N. K. Sanil

Holotype: Deposited in the parasite collections of Parasitology Laboratory, Department of Zoology, University of Calicut (Accession number: CF. 2.2).

Etymology: The name refers to the region (Malabar) from which the species was recorded for the first time

Period of collection: December, 1990

Prevalence: Five of 665 snails (0.75%) screened were infected

Cercariae emerged from snails during the daytime. Cercariae swim actively by vigorous lashing movements of their tails, interrupted by short periods of rest. While at rest, occupy the water column with their furcal rami spread out and body hanging down, at an angle with tail stem.

Description (Fig. 2a, b)

Pharyngeate, longifurcate furcocercous cercaria. Cercarial body elongate-oval, posterior half broader, measured

244.2–336.6 (291.1) $\times$ 122.1–168.3 (143.7); spinose; spines backwardly directed, evenly distributed. Cystogenous glands present. Tail terminal, spinose, robust with circular and longitudinal muscles; sensory hairs numerous; 297.0–343.2 (313.1) $\times$ 49.5–66.0 (56.8) in size. Furca shorter than tail, without constriction at base, aspinose except at its anterior third; finfolds present except at anterior fifth; measured 250.8–290.4 (267.5) $\times$ 29.7–33.0 (31.9). Fin folds supported by fine, ray-like structures which terminated in protruding setae. Caudal excretory canal bifurcates into furca and opens at its tip. Anterior organ terminal, longer than wide, measured 49.5–62.7 (55.2) $\times$ 46.2–56.1 (52.6). True ventral sucker absent, but a rudimentary structure present in its position.

Mouth terminal; prepharynx short; pharynx, muscular, pear-shaped, wider than long, 13.2–19.8 (16.7) $\times$ 16.5–23.1 (19.6) in size; oesophagus short; caeca long, wavy, extending to posterior end, caecal lumen encloses nine to 12 cells with granular contents and prominent nuclei. Penetration glands numerous, with short ducts, emptying on surface, mostly in anterior region.

Fig. 2 *Cercaria* sp. XVII Malabar n. sp. **a** Cercaria; **b** sporocyst

Table 2 Showing comparative characters and measurements of *Cercaria* sp. XVIII Malabar n. sp. and four related cercariae

Cercaria (Hosts)	<i>Cercariae indicae</i> XV Sewell 1922 (<i>Melanoides tuberculatus</i> , <i>M. lineatus</i>)	<i>Cercaria pendulata</i> Baugh 1954 (<i>Vivipara bengalensis</i>)	<i>Cercaria leytensis</i> no. 20 Ito 1977 (<i>Bellamyia philippinensis</i>)	<i>Cercaria laungakheraii</i> Srivastava and Saxena 1992 (<i>Bellamyia bengalensis</i>)	<i>Cercaria</i> sp. XVIII Malabar n. sp. (<i>Thiara tuberculata</i>)
Body	Aspinose, 316–368 × 72–158	Spinose, 264–330 × 148–205	Spinose, 220–250 (232) × 130–165 (145)	Spinose, 180–220 × 80–120	Elongate-oval, spinose, 244.2–336.6 (291.1) × 122.1–168.3 (143.7)
Tail	Aspinose, 210–368 × 53–70	Aspinose, 380–412 × 66	Spinose, 390–400 (392) × 40–50 (45)	Aspinose, 180–200 × 30–50	Spinose, 297.0–343.2 (313.1) × 49.5–66.0 (56.8)
Furcae	Aspinose, finfold present, 263–316	Spinose, 280–330	Anterior half spinose, 300–330 (310) × 18–20 (19)	Aspinose, 160–200 × 10–20	Anterior third spinose, finfold present, 250.8–290.4 (267.5) × 29.7–33.0 (31.9)
Anterior organ	58 × 46	—	35–45 (40) × 30–45 (39)	50–70 × 30–40	49.5–62.7 (55.2) × 46.2–56.1 (52.6)
Ventral sucker	Rudimentary	Rudimentary	Rudimentary	Rudimentary	Rudimentary
Prepharynx	Short	Long, narrow	Absent	Short	Short
Pharynx	Well developed, 22	Globular, 18–20 × 20–23	15–20 (17) × 18–20 (19)	Muscular, 20–27	Muscular, pear-shaped 13.2–19.8 (16.7) × 16.5–23.1 (19.6)
Oesophagus	Long	Broad, tubular	Moderate	Short	Short
Caeca	Wide, reach till excretory bladder	Till level of excretory bladder	Till level of excretory bladder	Till level of excretory bladder	Long, wavy, extending to posterior end
Excretory bladder	Trifoliate	Trifoliate	Cup-like	Transversely elongate	Transversely elongate
Caudal excretory canal	Till tip of furca	Till tip of furca	Till tip of furca	Till tip of furca	Opens at the tip of furca
Flamecell formula	2 [(12) + (3)] = 30	2 [(3 + 3 + 3) + (3 + 3 + (3))] = 36	2 [(12) + (3)] = 30	2 [(3 + 3 + 3) + (3 + 3 + (3))] = 36	2 [(3 + 3) + (3 + 3 + 3 + (3))] = 36

Numerous duct openings present in lateral fields in the anterior half of cercarial body. Duct openings seen in groups, exact number not discernible. Genital primordia an irregular mass of cells anterior to excretory bladder. Excretory bladder transversely elongate, kidney-shaped; two pairs of primary collecting vessels arise, one from anterior and other from antero-lateral margin of bladder. Anterior to rudimentary ventral sucker, inner pair of ducts unites to form a median excretory canal which runs forward and posterior to caecal bifurcation, divides into two. Each main canal of the outer pair ascends and lateral to caecal bifurcation, join the branch from the median canal on its side, to form a common canal which runs up to the posterior end of pharynx and ends blindly; canal on one side is always longer than the other. From the outer excretory canal, just posterior to union, a lateral branch arise, which runs down and divides into anterior and posterior collecting ducts near the equatorial plane of body. Excretory concretions present in median canal and its branches, a few ciliary patches were observed in lateral canals. Flame cell formula: $2 [(3 + 3) + (3 + 3 + 3 + 3)] = 36$.

Sporocyst (Fig. 2c)

Sporocysts develop in the digestive gland and stomach walls of infected *T. tuberculata*. Body elongated, pale, banded in appearance, with walls having a ridged structure; measured 1450.8–2012.4 (1680.6) × 218.4–265.2 (238.2); enclosed one to two cercariae, a few developing ones and germ balls.

Remarks

Among the longifurcate, pharyngeate, furcocercariae with rudimentary ventral sucker, the present cercaria needs to be compared with *Cercariae indicae* XV Sewell 1922, *Cercaria pendulata* Baugh 1954, *Cercaria leyteensis* no. 20 Ito 1977 and *Cercaria laungakheraii* Srivastava and Saxena 1992.

Table 2 provides comparison of characters and measurements of the studied cercaria with that of the above four forms. It differs from *C. indicae* XV in the spination of body, tail and furca and in having a short oesophagus; from *C. laungakheraii* in the spination of tail and furca and from *C. pendulata* in spination of tail. It differs from *C.*

pendulata, *C. leyteensis* no. 20 and *C. laungakheraii* in having finfolds on furcal rami. *C. leyteensis* no. 20 again differs from present form in the absence of prepharynx. Further, the present cercaria differs markedly from all the above forms in the organization of excretory system. In view of these differences, the above discussed cercaria appears to be a new species and is, therefore reported here as *Cercaria* sp. XIX Malabar n. sp.

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Author's contribution Dr. K. P. Janardanan designed and guided the study. Dr. N. K. Sanil carried out the survey, collected and studied the cercariae in detail. The manuscript was written by both the authors.

Compliance with ethical standards

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

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