Two new species of xiphidiocercariae from the apple snail *Pila virens* in Malabar, Kerala

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Abstract Two new species of xiphidiocercariae, *Cercaria* sp. XIV Malabar n. sp. and *Cercaria* sp. XV Malabar n. sp. were recovered from the apple snail *Pila virens* collected from freshwater bodies in the Malabar region of Kerala. *Cercaria* sp. XIV Malabar n. sp. was recovered from *P. virens* collected from Modakkallur in Kozhikode district and *Cercaria* sp. XV Malabar n. sp. from the same snail species collected from Payyanur in Kannur district. Sporocysts were also recovered from the infected snail hosts. The two new species of cercariae are described in detail and compared with related species to establish their systematic position.

Keywords Xiphidiocercaria · *Cercaria* · *Pila virens* · Malabar · Sporocyst

Introduction

Information on larval trematodes infecting the apple snail *Pila virens* (Lamarck) in India is limited to descriptions of 5 species by Ganapati and Rao (1969), Murty (1973), Janardanan and Shiny (1989) and Rajendran and Janardanan (1993). An extensive survey of *P. virens* inhabiting the freshwater bodies in the Malabar region of Kerala revealed the presence of two species of xiphidiocercariae in snails collected from Modakkallur in Kozhikode and Payyanur in Kannur districts. Comparison of the

morphological features of the present cercariae with those of related species revealed that these are distinctly different from the known species of xiphidiocercariae and are therefore, reported here as new species. The new species are designated by Roman numerals, followed by Malabar to indicate the region of collection.

Materials and methods

P. virens were collected from streams, canals, ponds and paddy fields in various localities of the Malabar region of Kerala, from April 1988 to August 1991. Infected snails were isolated, kept in beakers containing tap-water and the cercariae emerging from them were studied live, with or without vital staining using phase-contrast objectives of a Carl Zeiss Axioscope-2 plus Trinocular Research Microscope. Genital primordia were observed in lacto-acetic carmine-stained cercariae. Infected snails were later crushed and examined for intra-molluscan stages of development. Sporocysts recovered from the digestive glands of infected snails were studied after supravital staining with neutral red. Measurements were taken from 10 % formalin fixed specimens and are given in micrometres (µm) as the range followed by the mean in parentheses. Figures were drawn to scale with the aid of a camera lucida and details added free-hand from observations made on live specimens.

Results and discussion

Cercaria sp. XIV Malabar n. sp

Host: *Pila virens* (Lamarck)
Site of infection: Hepatopancreas

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Locality: Modakkallur in Kozhikode district, Kerala
 Period of collection: April 1988–August 1991
 Prevalence: Five of 525 (0.95 %) snails were infected

Cercariae emerge during night hours and exhibited negative phototaxis. Newly emerged cercariae swim vigorously by lashing movements of their tails, with intermittent resting periods.

Description (Fig. 1a, b)

Non-irrigulate xiphidiocercaria. Body elongate-oval, 90.0–124.5 (114.4) $\times$ 58.5–70.5 (64.7), spinose; spines minute, closely set, backwardly directed and uniformly distributed. Eight pairs of sensory hairs present on body. Tail highly contractile, aspinose, annulated, 79.5–120.0 (92.1) $\times$ 15.0–18.0 (16.6) in size. Oral sucker terminal, roughly round, 24.0–31.5 (28.7) $\times$ 27.0–33.0 (29.8), larger than ventral sucker. Ventral sucker post-equatorial, 15.0–18.0 (16.3) $\times$ 16.5–19.5 (17.9). Stylet dagger-shaped, inserted in antero-dorsal region of oral sucker, with fine tip, round base and well developed shoulder at about its anterior fourth; stylet wall reinforced except at posterior third; 13.5–15.0 (14.9) $\times$ 3.75–4.5 (4.4) in size. Mouth sub-terminal; prepharynx short; pharynx globular, wider

than long, 7.5–10.5 (8.5) $\times$ 7.5–10.5 (9.8); oesophagus short, narrow; caeca visible to a short distance, caecal tips not clear. Penetration glands 3 pairs, anterior 2 pairs pre-acetabular with ducts opening individually near tip of stylet, posterior pair para-acetabular with ducts opening near mid-region of stylet. Glands large, with irregular margins and round nuclei. Contents of anterior pair coarsely granular and posterior 2 pairs finely granular. Genital primordium 'C'-shaped, in close association with ventral sucker. Excretory bladder transversely elongate, bicornuate, thin-walled. Primary collecting vessels run from antero-lateral margins of bladder, take a convoluted course to posterior level of ventral sucker where they divide into anterior and posterior collecting tubules. Flame cell formula: 2 [(3 + 2+2) + (2 + 2+3)] = 28. Caudal excretory duct originates from posterior margin of excretory bladder, runs through tail and opens at its tip.

Sporocyst (Fig. 1c)

Sporocysts were recovered from the hepatopancreas of infected snail hosts. Body oval, saccular, 118.8–244.2 (175.4) $\times$ 99.0–145.2 (118.8), containing 1 to 2 cercariae, a few developing cercariae and several germ balls at various stages of development.

Remarks

Among the non-irrigulate xiphidiocercariae possessing spinose body and 3 pairs of penetration glands, the present form comes close to the descriptions of *Cercariae indicae* V by Sewell (1922), *Cercaria leyteensis* no. 26 by Ito (1977), *Cercaria premvatai* by Pandey and Singh (1986) and *Cercaria srivastavai* by Pandey and Singh (1986).

Table 1 gives a comparison of characters of these 4 species with that of the present form. The present cercaria differs from *C. indicae* V in having an aspinose tail and in the measurements of body and oral sucker. *C. leyteensis* no. 26 differentiates itself from the present form in possessing a refractile sac-like structure within the oral sucker, in the unequal distribution of spines and in the nature and arrangement of penetration glands. The present cercaria is distinct from *C. premvatai* in having a short prepharynx and in the measurements of body and ventral sucker. The position/nature and arrangement of penetration glands differentiates the present cercaria from *C. srivastavai*. Further, it differs from all the above cercariae in the shape of stylet and excretory bladder and in the number and arrangement of flame cells. Besides, the snail host of the present form is different from that of the other cercariae. In view of the reasons stated above, the present cercaria is considered as a new species and the name *Cercaria* sp. XIV Malabar n. sp. is proposed for it.

Fig. 1 *Cercaria* sp. XIV Malabar n. sp. **a** Cercaria, **b** stylet, **c** sporocyst

Table 1 Comparison of characters of *Cercaria* sp. XIV Malabar n. sp with related forms

<i>Cercaria</i> (hosts)	<i>Cercariae indicae</i> V Sewell (1922) (<i>Melanoides tuberculata</i> , <i>Digoniostoma ceraneopoma</i>)	<i>Cercaria leytensis</i> no. 26 Ito (1977) (<i>Bellamyia philippinensis</i>)	<i>Cercaria premvatai</i> Pandey and Singh (1986) (<i>Bellamyia bengalensis</i>)	<i>Cercaria srivastavai</i> Pandey and Singh (1986) (<i>Bellamyia bengalensis</i>)	<i>Cercaria</i> sp. XIV Malabar n. sp. (<i>Pila virens</i>)
Body	Elongate, spinose, 140–220 × 66–114	Spinose, 102–117 (110) × 48–60 (55)	Spinose, 100–180 × 50–80	Spinose, 80–100 × 40–60	Uniformly spinose, 90.0–124.5 (114.4) × 58.5–70.5 (64.7)
Tail	Spinose, 70–140	Aspinose, 50–100 (70) × 15–22 (19)	Aspinose, 50–170 × 20–40	Aspinose, 90–120 × 20–30	Aspinose, annulate, 79.5–120.0 (92.1) × 15.0–18.0 (16.6)
Oral sucker	36	27–33 (30) × 25–30 (28)	20–30	20–30	24.0–31.5 (28.7) × 27.0–33.0 (29.8)
Ventral sucker	18	14–17 (15) × 15–20 (17)	8–12	10–20	Post equatorial, 15.0–18.0 (16.3) × 16.5–19.5 (17.9)
Stylet	14	15–18 (17)	Spear-shaped	Spear-shaped	Dagger-shaped, 13.5–15 (14.9)
Prepharynx	Absent	Short	Long, narrow	Short	Short
Pharynx	Globular	Spherical, 7–12 (10)	Globular, 10–20	Globular, 8–10	Globular, 7.5–10.5 (8.5) × 7.5–10.5 (9.8)
Oesophagus	Long	Long	Short	Long	Short, narrow
Caeca	Short, narrow	Up to the middle of VS	Short	Up to the middle of VS	Short, narrow
Excretory bladder	'V'-shaped, bilobed	'Y'-shaped	'U'-shaped	'V'-shaped	Transversely elongate, bicornuate
Flame cell formula	2 × 6 × 1 = 12	2 [(2 + 2) + (2 + 2)] = 16	2 [(3 + 3) + (3 + 3)] = 24	2 [(3 + 3) + (3 + 3)] = 24	2 [(3 + 2 + 2) + (2 + 2 + 3)] = 28

***Cercaria* sp. XV Malabar n. sp.**

Host: *Pila virens* (Lamarck)

Site of infection: Hepatopancreas

Locality: Payyanur in Kannur district, Kerala

Period of collection: April 1988–August 1991

Prevalence of infection: Three of 525 (0.57 %) snails were infected

Cercariae emerged during the evening hours with maximum emergence from 5 to 7 pm. Cercaria swims by lashing movements of its tail; prolonged swimming activity is interrupted by brief periods of rest.

Description (Fig. 2a, b)

Non-irrigulate xiphidiocercaria. Body elongate-oval, 70.5–91.5 (83.7) × 34.5–45.0 (41.0), spinose; spines small, backwardly directed and uniformly distributed except on ventral sucker, where they are directed toward the center of sucker. Two pairs of sensory hairs present on body. Tail highly contractile, aspinose, annulated, 63.0–88.5 (73.7) × 12.0–13.5 (12.3); a pair of sensory hairs present at mid region. Oral sucker subterminal, roughly round, 18.0–22.5 (21.4) × 19.5–22.5 (21.5); larger than ventral sucker. Ventral sucker roughly round, post-equatorial, 12.0–15.0 (12.9) × 10.5–13.5 (11.7). Stylet spear-shaped, inserted in antero-dorsal region of oral sucker, with pointed tip, broad, round base and moderately developed shoulder region at about its anterior fourth; 12.0–13.5 (13.4) × 2.9–3.0 (3.0); stylet wall reinforced except at base. Mouth subterminal; prepharynx absent; pharynx globular, muscular, 6.0–7.5 (6.5) × 6.0–7.5 (7.1); oesophagus narrow; caeca not observed. Penetration glands 3 pairs, lateral to ventral sucker, ovoid, with round nuclei and granular contents; gland size increases from anterior to posterior; gland ducts open individually near shoulder of stylet. Genital primordium 'C'-shaped, closely associated with ventral sucker. Excretory bladder 'V'-shaped, thin-walled. Primary collecting vessels run from antero-lateral margins of bladder, take a convoluted course to the level of ventral sucker where they divide into anterior and posterior collecting tubules. Flame cell formula, 2 [(2 + 2+2) + (2 + 2+2)] = 24. Caudal excretory duct originates from posterior margin of excretory bladder, runs through tail and opens at its tip.

Sporocyst (Fig. 2c)

Sporocysts were recovered from the hepatopancreas of infected snail hosts. Body elongate, saccular, 352–670 (474.8) × 132–260 (190.4), containing 2 to 3 cercariae,

Fig. 2 *Cercaria* sp. XV Malabar n. sp. **a** cercaria, **b** stylet, **c** sporocyst

several developing cercariae and a few germ balls at different stages of development.

Remarks

The present non-irrigulate xiphidiocercaria with spinose body and 3 pairs of penetration glands needs comparison with the descriptions of *Cercariae indicae* V by Sewell (1922), *Cercaria leyteensis* no. 15 by Ito (1977), *Cercaria leyteensis* no. 26 by Ito (1977) and *Cercaria varanasiensis* V by Singh and Agarwal (1995).

From a comparison of characters of the present form with those of the above 4 species presented in Table 2, it is clear that the present form could be distinguished from *C. indicae* V in having an aspinose tail and in the absence of caeca; from *C. leyteensis* no. 26 in the absence of refractory bodies in the oral sucker and from all others except *C. leyteensis* no. 15 in the absence of caeca. In the shape of stylet, it differs from all the above cercariae except *C. indicae* V. Further, the present form is distinct from the others in size of body and suckers, arrangement of penetration glands and in the nature of their contents and in the number and arrangement of flame cells. Besides, the snail

Table 2 Comparison of characters of *Cercaria* sp. XV Malabar n. sp with related forms

Cercaria (hosts)	<i>Cercariae indicae</i> V Sewell (1922) (<i>Melanoides tuberculata</i> , <i>Digoniostoma cerameopoma</i>)	<i>Cercaria leytensis</i> no 15 Ito (1977) (<i>Melanoides tuberculata</i> , <i>Antimelania dactylus</i> , <i>A. asperata</i>)	<i>Cercaria leytensis</i> no. 26 Ito (1977) (<i>Bellamya philippinensis</i>)	<i>Cercaria varanasiensis</i> V Singh and Agarwal (1995) (<i>Lymnea acuminata</i>)	<i>Cercaria</i> sp. XV Malabar n. sp. (<i>Pila virens</i>)
Body	Elongate, spinose, 140–220 × 66–114	Spinose, 85–115 (100) × 67–85 (77)	Spinose, 102–117 (110) × 48–60 (55)	Spinose, 136–144 × 102–108	Spinose, 70.5–91.5 (83.7) × 34.5–45.0 (41.0)
Tail	Spinose, 70.0–140.0	Aspinose, 70–90 (80) × 17–22 (19)	Aspinose, 50–100 (70) × 15–22 (19)	Aspinose, 57–66 × 12–15	Aspinose, 63.0–88.5 (73.7) × 12.0–13.5 (12.3)
Oral sucker	36	25–32 (28)	27–33 (30) × 25–30 (28)	36–39	18–22.5 (21.4) × 19.5–22.5 (21.5)
Ventral sucker	18	16–20 (18)	14–17 (15) × 15–20 (17)	22.7–30	12.0–15.0 (12.9) × 10.5–13.5 (11.7)
Stylet	14	16–20 (18) × 3.5	15–18 (17)	12–15	12.0–13.5 (13.4) × 2.9 – 3.0 (3.0)
Prepharynx	Absent	Absent	Short	Absent	Absent
Pharynx	Globular	Globular, 6–8 (7) × 7–9 (8)	Spherical, 7–12 (10)	Globular, 9–12	Globular, 6–7.5 (6.5) × 6–7.5 (7.1)
Oesophagus	Long	Short	Long	Short, broad	Narrow
Caeca	Short, narrow	Absent	Up to the middle of VS	Short	Absent
Excretory bladder	'V'-shaped, bilobed	'V'-shaped	'Y'-shaped	Bicornuate	Bicornuate, 'V'-shaped
Flame cell formula	$2 \times 6 \times 1 = 12$	$2 [(2 + 2) + (2 + 2)] = 16$	$2 [(2 + 2) + (2 + 2)] = 16$	$2 [(2 + 2) + (2 + 2)] = 16$	$2 [(2 + 2 + 2) + (2 + 2 + 2)] = 24$

host of the present form is also different from that of the other cercariae. As the present cercaria could not be identified with any of the known cercariae, it is considered as new and named *Cercaria* sp. XV Malabar n. sp.

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Compliance with ethical standards

Conflict of interest N. K. Sanil reports Grants from Council of Scientific and Industrial Research (CSIR), New Delhi, during the conduct of the study.

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