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VERSITA

Studies on the life-cycle of *Uvulifer iruvettiensis* sp. nov. (Digenea: Diplostomidae)

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Abstract

The life-cycle stages of a new species of *Uvulifer*, *U. iruvettiensis* sp. nov. infecting the white-throated kingfisher *Halcyon smyrnensis* (Linnaeus) in Kerala, India are described. The new species is described in detail, and compared with its related species. The furcocercous cercariae emerged from the planorbid snail *Indoplanorbis exustus* (Deshayes). Metacercariae were found below the skin of tadpoles of *Euphlyctis cyanophlyctis* (Schneider). *H. smyrnensis* became infected when fed on tadpoles containing infective metacercariae. Pre-patent period is 10 days. Successive stages in the development of metacercariae and adults were followed in the laboratory.

Keywords

Uvulifer iruvettiensis, life-cycle, *Halcyon smyrnensis*, cercaria, *Indoplanorbis exustus*, metacercaria, tadpoles, *Euphlyctis cyanophlyctis*

Introduction

Yamaguti (1934) erected the genus *Uvulifer* with *U. gracilis* from the kingfisher *Ceryle lugubris* (Temminck) in Japan as the type. Yamaguti (1971) listed 9 species under the genus *Uvulifer*. Since then, 8 species have been added to this genus by several workers all over the world (Oshmarin 1970; Baer 1971; Dubois 1977, 1985, 1988; Mishra and Gupta 1980). Of the 17 valid species, 6 are from Indian birds. These are, *U. ceryliformes* (Vidyarthi, 1938) Bhalerao, 1948, *U. stunkardi* (Pande, 1938) Bhalerao, 1942, *U. cochlearis* (Verma, 1936) Dubois, 1944, *U. mehrai* Chatterji, 1956, *U. giriensis* Mishra et Gupta, 1980 and *U. chandigarhensis* Mishra et Gupta, 1980. The life-cycle of only one species of *Uvulifer*, *U. ambloplitis* (Hughes, 1927) Dubois, 1938 has been established (Hunter and Hunter 1930, 1934, 1935; Hoffman and Putz 1965).

During the present investigation on the digenean fauna of piscivorous birds in Kerala, India, we came across a species of *Uvulifer* in the intestine of the white-throated kingfisher *Halcyon smyrnensis* (Linnaeus) collected from Iruvetty and Puthur Pallikkal in Malappuram and Cheruvady in Kozhikkode districts. Further studies proved that it is a new species and named *Uvulifer iruvettiensis* sp. nov. Studies on the cercarial fauna of freshwater snails in these localities revealed that a furcocercous cercaria emerged from the planorbid snail *Indoplanorbis exustus* (Deshayes) during the monsoon season (June-September). These cercariae encysted in tadpoles of the frog *Euphlyctis cyanophlyctis* (Schneider) of the same locality. The infective metacercariae when fed to nestlings of *H. smyrnensis* developed into adults of *U. iruvettiensis* in its intestine within 10 days. The life-cycle stages, from cercariae to egg-producing adults, were successfully established in the laboratory. This forms the first report on the life-cycle of a *Uvulifer* sp. from India; it is also the second report on the life-cycle of a species of *Uvulifer*.

Materials and methods

The freshwater snails *Pila virens* (Lamarck), *Bellamya dissimilis* (Mueller), *Paludomus tanschauricus* (Gmelin), *Lymnaea luteola* Lamarck, *Thiara tuberculata* (Mueller) and *Indoplanorbis exustus* (Deshayes), which were the only snail species present in the paddy fields, ponds and other water bodies at Iruvetty, Puthur Pallikkal and Cheruvady, were collected from January, 2006 to March, 2007. The snails were kept in

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separate beakers containing tap water and screened for infection with furcocercous cercariae. *I. exustus* was the only snail species from which furcocercous cercariae emerged. The cercariae emerging from the snails were studied live after supravitally staining with neutral red. Genital primordia were observed in lacto-acetic carmine-stained cercariae. Measurements were made on heat-killed specimens. A few infected snails were later crushed and examined for intra-molluscan stages of development. Sporocysts recovered from the digestive glands of infected snails were studied live with or without vital staining.

The water bodies in which the infected snails inhabited were searched for the suspected second intermediate hosts of the trematode. Tadpoles of *Euphlyctis cyanophlyctis* (Schneider) and *Hoplobatrachus tigerinus* (Daudin), and fishes like *Rasbora daniconius* (Hamilton), *Puntius filamentosus* (Valenciennes), *P. sarana* (Hamilton), *Gara mullya* (Sykes), *Mastacembelus armatus* (Lacepede), *Mystus malabaricus* (Jerdon), *Xenentodon cancila* (Hamilton) and *Aplocheilus lineatus* (Valenciennes) were collected from these water bodies and examined for natural infection with metacercariae. The black pigmented metacercariae, suspected to be that of *Uvulifer* species, were found only in tadpoles of *E. cyanophlyctis*. For experimental infection studies, infection-free tadpoles of *E. cyanophlyctis*, collected from a tank in the Calicut University Campus and proved to be free of natural infection with metacercariae of *Uvulifer* sp., were exposed to newly emerged cercariae and examined at intervals to observe metacercarial development.

Piscivorous birds present in the study area were collected from the roosting sites and premises of water bodies and examined for natural infection with adult flukes. The birds examined were *Phalacrocorax niger* (Vieillot), *Ardeola grayii* (Sykes), *Egretta garzetta* (Linnaeus), *Bubulcus ibis* (Linnaeus), *Anastomus ocitans* (Boddaert), *Milvus migrans* (Boddaert), *Alcedo atthis* (Linnaeus) and *Halcyon smyrnensis* (Linnaeus). The present fluke was found infecting only *H. smyrnensis*.

In order to obtain adult flukes experimentally, infective metacercariae were fed to 1-week old nestlings of *H. smyrnensis*. The development of adult flukes was traced by examining the experimental birds periodically. Sixteen wild caught *H. smyrnensis* were also examined for the presence of natural infections by the adult flukes.

Metacercariae and adults obtained were studied live, with or without supravital staining using neutral red. Those used for permanent preparations were fixed in AFA under a slight cover-glass pressure and stained in alum carmine following the procedure outlined by Cantwell (1981).

Descriptions of larval and adult stages are based on the measurements of 15 specimens. All measurements are given in micrometres, as the range followed by the mean in parentheses. Figures were drawn with the aid of a camera-lucida and details were added free-hand from observations made on live specimens.

Life-cycle

Egg (Fig. 1a): Eggs yellowish-brown, ovoid, operculate, 92–99 (96) x 59–73 (69). Attempts made to develop miracidia were unsuccessful.

Sporocyst (Fig. 1b): Sporocysts were recovered from the hepatopancreas of *I. exustus*. Body reddish-brown, elongate, thread-like, 789–1045 (987) x 26–50 (39), containing 8–9 cercariae and a few germ balls at different stages of development.

Cercaria (Fig. 1c): Natural infections with cercariae were found in 30 of 1400 (2.14%) *Indoplanorbis exustus* collected from Iruvetty, Puthoor Pallikkal and Cheruvady. Other snail species collected from these localities were free from this infection. Cercariae emerged during the night hours. Cercariae exhibited characteristic swimming behaviour with short spells of swimming activity followed by prolonged resting periods. During resting cercariae remain suspended in the water column with the body downward, tail stem turned upward and furcae diverged at an angle of about 60° to the tail stem.

Description: Longifurcate, pharyngeate, ocellate, distome cercaria. Body elongate-oval, 116–159 (124) x 53–59 (56), with pear-shaped anterior organ. Anterior organ highly muscular, 32–46 (38) x 20–32 (28), with 8–11 rows of prominent, backwardly directed spines in its anterior half. Cephalic glands two, on either side of oral cavity in anterior organ. Pre-oral spines 11, in three transverse rows; first row near mouth with 3 spines, and second and third rows with 4 spines each. Two bands of densely distributed spines encircling the body present at the posterior end of anterior organ; spines in between the bands sparsely distributed. Rest of body devoid of spines. Ventral sucker rudimentary, at the end of middle third of body. A pair of non-pigmented eye spots present at mid region of body. Tail stem aspinose, 175– 242 (223) x 20–34 (28), provided with 14 pairs of long sensory hairs. Furcae 165–198 (175) x 23–45 (32) in size, with longitudinal rows of spines; two short sensory hairs on each furca.

Mouth subterminal; prepharynx short; pharynx globular, 11–15 (13) in diameter; oesophagus and caeca not discernible. Penetration glands 3 pairs, on either side of ventral sucker; each gland with finely granular contents and large nucleus; ducts of penetration glands on each side form a bundle directed anteriorly, pass over anterior organ, and open at anterior margin through 3 pores guarded by spines. Cystogenous glands numerous, beneath tegument. Genital primordium represented by conical mass of cells located just anterior to excretory bladder. Excretory bladder ovoid, at posterior end of body. Flame cell formula: 2 (2) + (2+2+2) + (2) = 20, with posterior 2 pairs in anterior third of tail stem. Caudal excretory duct runs through tail stem, bifurcates and runs into each furca and opens out mid-furcally.

Metacercaria (Fig. 2a): Natural infections with metacercariae were found below the skin of tadpoles of *Euphlyctis cyanophlyctis* collected from the same water bodies where infected snails were present. The other suspected second intermediate hosts collected from these water bodies were free

from the metacercarial infection. Prevalence of infection was 66% (33 of 50 tadpoles examined were infected). Intensity of infection varied from 25 to 40. Infection could be established experimentally in tadpoles of *E. cyanophlyctis*. In the laboratory, the tadpoles were exposed to newly emerged cercariae. Cercariae actively penetrated the skin of tadpoles within 1h after exposure. Metacercariae enclosed in a thin, round, transparent cyst wall were observed after 24 hours. On the 10th day

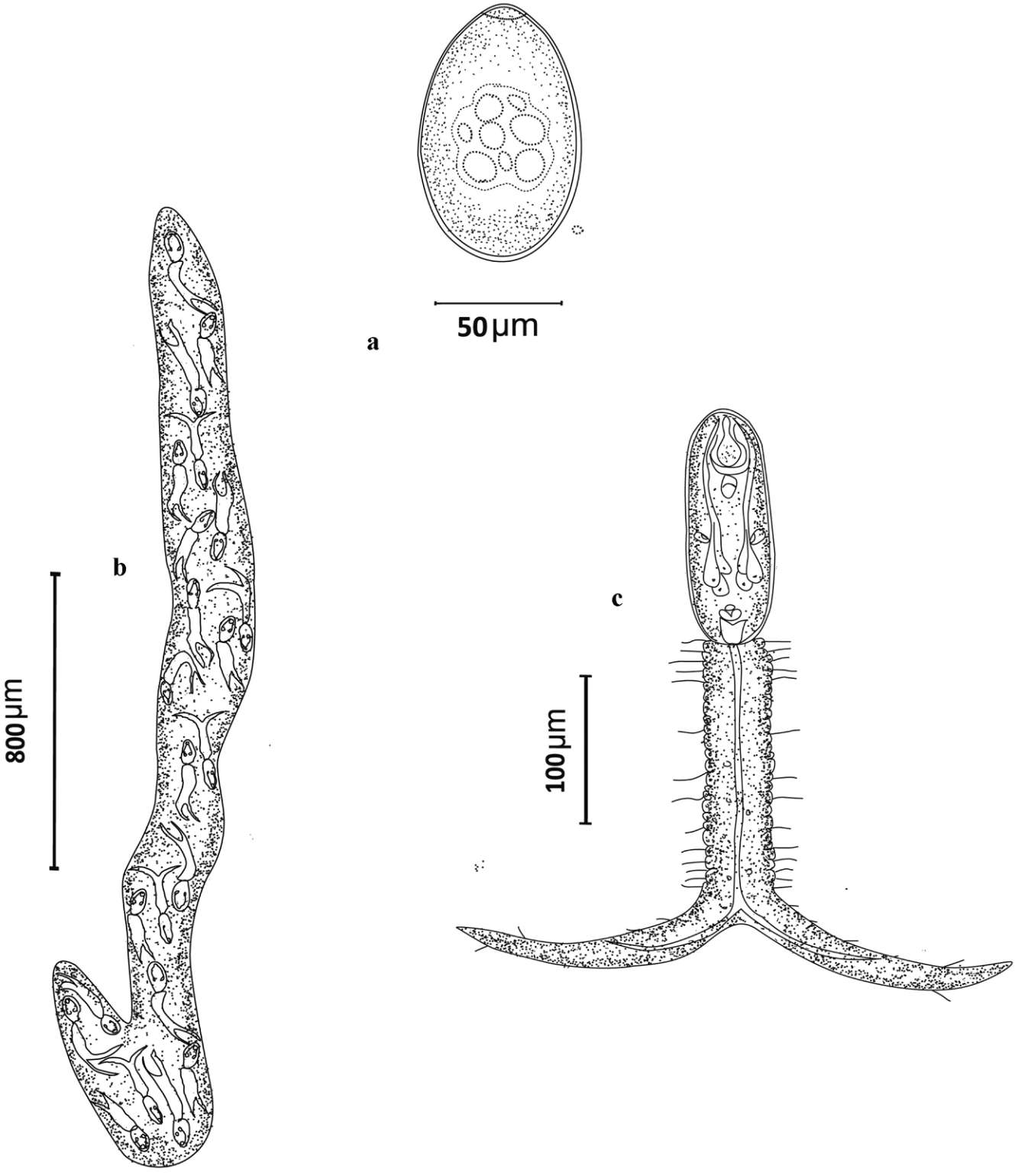

Fig. 1. Life-cycle stages of *Uvulifer iruvettiensis* sp. nov; **a –** egg, **b –** sporocyst, **c** –cercaria

post-exposure, the cysts became larger and ovoid, measured 285–320 (296) x 169–250 (234). Metacercariae recovered after 22 days post-exposure were infective to the definitive host. All the tadpoles exposed to cercariae were infected; as many as 90 cysts could be obtained from a single tadpole. These metacercariae were identical to those obtained from natural infections.

Description: Cysts brownish-black, round to ovoid, 231–374 (275) x 218–262 (237). Cyst wall bilayered, with outer, black-pigmented layer and inner, thin, tough, hyaline layer. Metacercaria lies folded inside cyst cavity. Metacercarial body, 501–593 (547) long, divisible in to distinct foliaceous forebody, 301–362 (329) x 208–231 (214), and conical hindbody, 193 –231 (212) x 172–207 (198). Oral sucker ovoid, terminal, 77–92 (83) x 54–83 (77). Ventral sucker round, 31–46 (39) in diameter. Holdfast organ round, 77–93 (85), immediately posterior to ventral sucker. Prepharynx very small; pharynx globular, muscular, 31–39 (33); oesophagus 65–77 (68) long; intestinal caeca slender, reaching to anterior margin of excretory bladder. Developing testes two, tandem, round to ovoid, at posterior end of hind-body; anterior testis 61–77 (75); posterior testis 65–78 (76). Ovary in the form of group of cells located anterior to testis. Excretory bladder V-shaped. Reserve excretory system extensively developed.

Development of adults

Development of flukes was observed in experimentally infected nestlings of *Halcyon smyrnensis*. Tadpoles with infective metacercariae were fed to the birds. Excysted metacercariae were observed in the intestine within 12 h post-infection and were without any marked changes from encysted metacercariae in structural organization. Four-day-old immature adults showed slight increase in size of fore- and hindbody, suckers, and reproductive organs. Six-day-old specimens (Fig. 2b) became larger with forebody measuring 314–342 (324) x 281–308 (298), hindbody 424–539 (514) x 231–315 (287), anterior testis 67–87 (78) x 80–106 (95), posterior testis 89–106 (98) x 63–78 (72) and ovary 54–73 (65) x 54–89 (72). At 10 days post-infection, flukes are fully mature and contained one egg in the uterus. The experimentally developed flukes were identical to those recovered from naturally infected birds.

Adult (Fig. 3): Natural infections with the adults of the present fluke were found in 12 of 16 (75%) *H. smyrnensis*. The intensity of infection varied from 50 to 100 (85). All other bird species collected from the study area were negative for this infection.

Description: Body distinctly bipartite, 790–1669 (1139) in length. Forebody cochleariform, 343–501 (413) x 312–390 (352); hindbody claviform, 770–1248 (963) x 223– 362 (291) long. Ratio of forebody length to hindbody length 1 : 2.2–2.96 (2.51). Tegument unarmed. Oral sucker terminal, round, 56–99 (77). Ventral sucker round, 33–50 (39), anterior to middle line of forebody. Distance between oral sucker and ventral sucker 55–122 (83). Sucker width ratio 1:0.46–0.58 (0.51). Holdfast organ located immediately posterior to ventral sucker, nearly round, 76–116 (103) x 85–149 (113), lobed, with a median longitudinal slit. Ratio of body length to holdfast organ length 1:0.06–0.09 (0.08). Ratio of forebody length

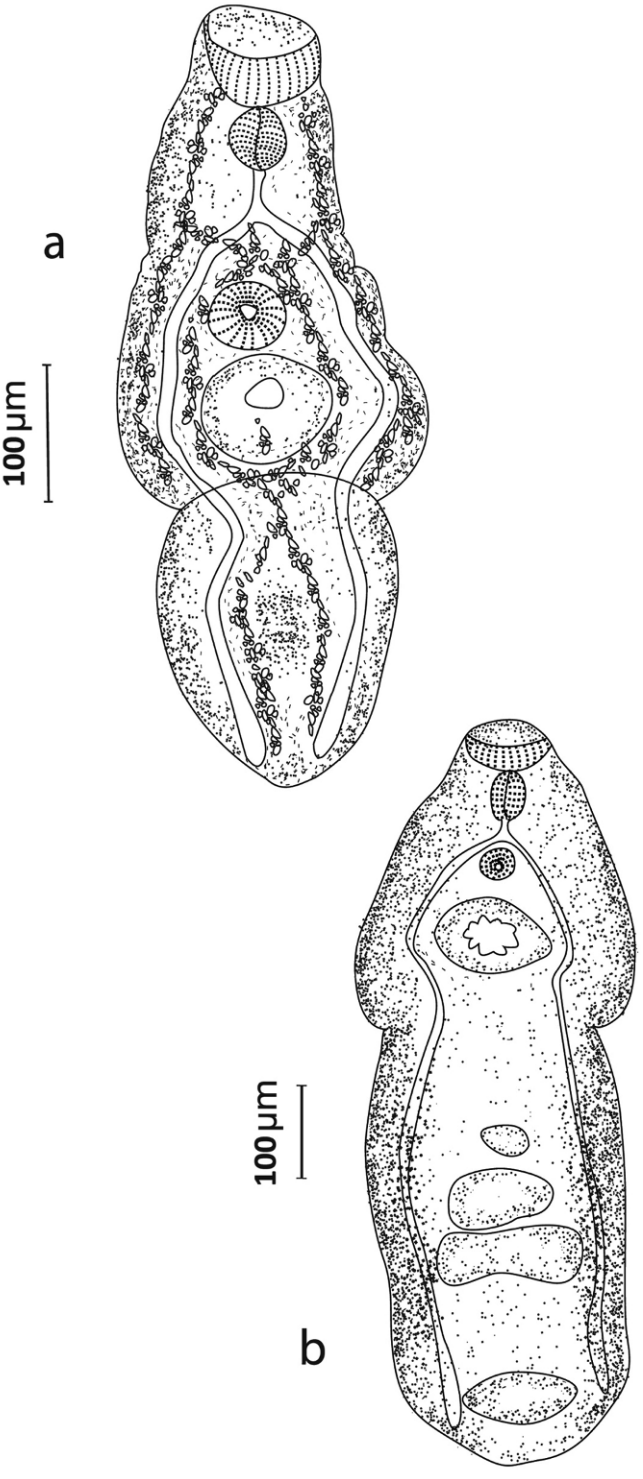

Fig. 2. Excysted metacercaria of *Uvulifer iruvettiensis* sp. nov. (**a**), six-day-old immature adult (**b**)

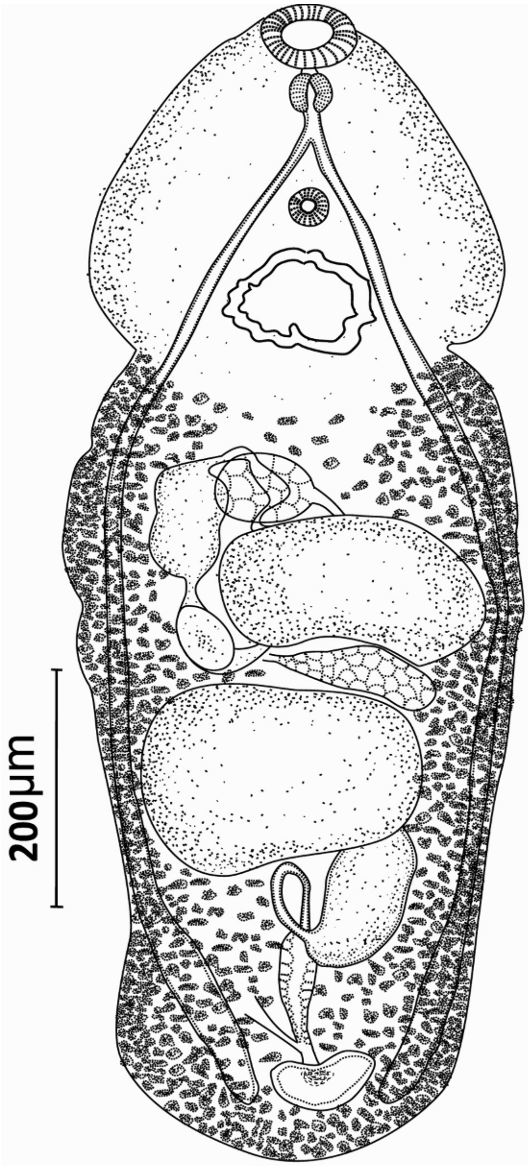

Fig. 3. Mature adult of *Uvulifer iruvettiensis* sp. nov.

to holdfast organ length 1 : 0.19–0.35 (0.27). Prepharynx very small, 3–8 (6); pharynx ovoid, muscular, 33–50 (37) x 26–43 (35); oesophagus short, narrow, 13–39 (20) long; intestinal caeca long, reaching to hind end of body. Ratio of oral sucker length to pharynx length 1 : 0.33–0.6 (0.47).

Testes tandem, at middle-third of hindbody; anterior testis dorsoventrally elongated, 70–193 (130) x 109–270 (176); posterior testis transversely elongated, 76–246 (148) x 177– 231 (198). Seminal vesicle posterior to testis, saccular, 77–231 (184) x 100–184 (122), opens into the muscular ejaculatory pouch. Copulatory bursa with protrusible genital cone half enclosed by prepuce-like fold. Hermaphroditic duct opens at the apex of genital cone.

Ovary median, ovoid, pre-testicular, 69–100 (84) x 96–139 (122). Mehlis' gland complex and vitelline reservoir intertes-

Table I. Comparison of characters of *Uvulifer stunkardi* (Pande, 1938) Bhalerao, 1942, *U. cerylou* Dollfus, 1950, *U. chandigarhensis* Mishra et Gupta, 1980 and *U. iruvettiensis* sp. nov.

Character	*Uvulifer stunkardi*	*U. cerylou*	*U. chandigarhensis*	*U. iruvettiensis* **sp. nov.**
Body length	820–1650	530–730	1950–2520	790–1669 (1139)
Forebody	290–460 x 200–410	150–210 x 160–260	580–720 x 300–470	323–468 x 312–390
Hindbody	530–1190 x 230–540	380–520 x 130–212	1360–1800 x 320–430	770–1248 x 343–501
Oral sucker	Ovoid, 45–100 x 66–120	Ovoid, 33–38 x 38–58	Ovoid, 45–65 x 60–98	Round, 56–99
Ventral sucker	Ovoid, 30–50 x 37–61	Ovoid, 11–18 x 20–29	Round, 35–52	Round, 33–50
Holdfast organ	Nearly round, 70–130 x 70–140, without peripheral glands	Nearly round, lobed 42–82 x 42–95 with thick peripheral glands	Nearly round, 80–144 x 85–160 with rudimentary peripheral glands	Nearly round, lobed, 76–116 x 85–149 without peripheral glands
Pharynx	28–50 x 28– 45	16–21 x 14–19	30–45 x 30–48	Ovoid, 33–50 x 26 – 43
Oesophagus	Short, 30–70	Short, 18–36	Long, 100	Short, 13–39
Anterior testis	Ovoid 120–270 x 150–280	Dorsoventrally elongated 96 x 142–165	Dorsoventrally elongated 315–355 x 275–325	Dorsoventrally elongated 70–193 x 109–270
Posterior testis	Ovoid 100–330 x 100–360	Transversely elongated 40–65 x 70–200	Transversely elongated 320–355 x 325–350	Transversely elongated 76–246 x 177–231
Ovary	Nearly round, 40–100 x 50–110	Ovoid, 36–42 x 47– 63	Round, 132–145	Ovoid, 69–100 x 96–139
Eggs	Three to four in number 84–98 x 49–57	One in number 79–91 x 51–62	Three to four in number 45–50 x 20–30	One in number 92–99 x 59–73
Host	*Halcyon smyrnensis* (Linnaeus)	*Ceryle rudis* (Temminck)	*H. smyrnensis* (Linnaeus)	*H. smyrnensis* (Linnaeus)

ticular. Vitellarium follicular, confined to lateral fields of hindbody, extending from intersegmental constriction to posterior end of body. Uterus with one egg.

Type-host: *Halcyon smyrnensis* (Linnaeus).

Site: Intestine.

Type-locality: Iruvetty, Malappuram district, Kerala, India.

Additional localities: Puthur pallikkal, Malappuram district and Cheruvady, Kozhikkode district, Kerala, India.

Period of collection: January, 2006 to March, 2007.

Prevalence: Twelve of 16 *H. smyrnensis* examined were infected.

Intensity: 50–100.

Holotype: Deposited in the Department of Zoology, University of Calicut, Kerala, India NO. Z./Par./Dig.-2007 1a.

Paratypes: Z./Par./Dig.-20071b-d.

Snail host: *Indoplanorbis exustus* (Deshayes).

Second intermediate host: Tadpoles of *Euphlyctis cyanophlyctis* (Schneider).

Discussion

The digenetic trematode infecting the white-throated kingfisher *Halcyon smyrnensis* has cochleariform forebody, claviform hind body which is much longer than forebody, ventral sucker smaller than oral sucker, tandem testes, pretesticular ovary, vitellarium in hind body, seminal vesicle followed by muscular ejaculatory pouch and copulatory bursa with protrusible genital cone half enclosed by preputial fold. These characters amply justify its inclusion in the genus *Uvulifer* Yamaguti, 1934 of the family Diplostomidae Poirier, 1886 (Niewiadomska 2002). As far as is known, the genus *Uvulifer* is found restricted to kingfishers (Alcidines). Five species have so far been reported from *H. smyrnensis*: *U. ceryliformes*, *U. stunkardi*, *U. cochlearis*, *U. mehrai* and *U. chandigarhensis*. The present species is also recovered from *H. smyrnensis*, and mixed infection with other species of *Uvulifer* was not observed in the 16 birds examined as far as we could see. Similarly the present species is not found infecting the other 7 bird species examined during the course of the present investigation.

The present *Uvulifer* species has well developed ventral sucker, holdfast organ located behind ventral sucker, tandem testes at middle-third of hindbody and vitellaria confined to lateral fields extending from intersegmental constriction to posterior end of body in addition to other characters. Based on these characters the present form comes close to *U. stunkardi* (Pande, 1938) Bhalerao, 1942, *U. cerylou* Dollfus, 1950 and *U. chandigarhensis* Mishra et Gupta, 1980 among the 17 valid species of *Uvulifer*. The remaining 14 species are distinctly different from the present fluke in one or more of the above characters.

A comparative study of characters of *U. stunkardi*, *U. cerylou*, *U. chandigarhensis* and the present species (Table I) revealed that *U. stunkardi* is different from the present form in having ovoid and larger suckers and testes, longer oesophagus, and uterus with 3–4 relatively smaller eggs. *U. cerylou* is smaller in size and shape of body and suckers and it has comparatively smaller pharynx, testes, ovary and eggs. Besides, its holdfast organ is smaller and is provided with thick peripheral glands whereas that of the present form is larger and devoid of peripheral glands. *U. chandigarhensis* on the other hand has very large body and testes, ovoid oral sucker, round ovary and longer oesophagus. Further, it has holdfast organ which is provided with rudimentary peripheral glands and its uterus contains 3–4 eggs, which are almost half in size when compared with that of the present form. Based on the facts stated above, the present fluke differs significantly from all the known species of *Uvulifer* and is, therefore, reported here as a new species and named *Uvulifer iruvettiensis* sp.nov., after the type-locality.

The life-cycle of only one species of *Uvulifer*, *U. ambloplitis* (Hughes, 1927) Dubois, 1938 has been elucidated (Hunter and Hunter, 1930, 1934, 1935; Hoffman and Putz 1965). It exhibited a three-host life-cycle with *Helisoma trivolvis* (Say) (now *Planorbella trivolvis*) and *H. companulatum* (Say) (now *P. complanulata*) as the snail hosts, fishes like, *Enneacanthus obesus* (Girard) and *Lepomis macrochirus* Rafinesque as the second intermediate hosts and the belted kingfisher, *Megaceryl alcyon alcyon* (Linnaeus) as the definitive host. The life-cycle from egg to egg-producing adults took 140 days to complete: miracidia came out of the eggs in 3 weeks; cercariae emerged from the snails in 42 days; metacercariae took 22–27 days in fish hosts to become infective, and developed into mature adults in 50 days in the intestine of the definitive host. The present fluke also has a three-host life-cycle pattern showing differences with regard to the hosts involved and the structural details of different stages in the life-cycle. The longifurcate cercariae emerged from the planorbid snail *Indoplanorbis exustus* encysted below the skin of tadpoles of *Euphlyctis cyanophlyctis*. Metacercariae became infective in 22 days and took only 10 days to become mature adults in the intestine of *Halcyon smyrnensis.*

The cercaria recovered during the present study from *I. exustus* agrees fully with *Cercaria* sp. VIII Malabar, described by Vasandakumar and Janardanan (2006) from the same snail species in Kannur district in all respects and are therefore identical. Other snail species of the habitat were found to be free from infection with the cercariae. The present metacercaria needs to be compared with the metacercaria of *Uvulifer ambloplitis* infecting freshwater fishes (Hunter and Hunter 1930, 1934, 1935; Hoffman and Putz 1965). It is evident from a comparative study that the present metacercaria is different from that of *U. ambloplitis* in the size of body, suckers and holdfast organ, in the nature and distribution reserve excretory system and in other minor details. Besides, it is recovered from tadpoles of *E. cyanophlyctis* only where as the other is reported from freshwater fishes. It is to be noted that the freshwater fishes collected from the water bodies of the study area were negative to the present metacercaria. Adult

flukes were found only in the intestine of the bird *H. smyrnensis*, and all other bird species of the area were negative to this infection. This suggests that *Uvulifer iruvettiensis* sp. nov. exhibits strict specificity at the level of first and second intermediate hosts and the definitive host.

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