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Sabhyata

Department of Zoology,
DAV College, Kanpur,
Uttar Pradesh, India

Pankaj Tandon

Department of Zoology,
DAV College, Kanpur,
Uttar Pradesh, India

MK Sinha

Department of Zoology,
DAV College, Kanpur,
Uttar Pradesh, India

Chandra Saurabh

Department of Zoology,
DAV College, Kanpur,
Uttar Pradesh, India

Corresponding Author:

Sabhyata

Department of Zoology,
DAV College, Kanpur,
Uttar Pradesh, India

Occurrence of a new trematode parasite species *Opisthorchis varunai* of family Opisthorchiidae in fresh water fish *Wallago attu*

Sabhyata, Pankaj Tandon, MK Sinha and Chandra Saurabh

Abstract

During the microscopic anatomical study of a fresh water silurid fish, *Wallago attu*, a new trematode species *Opisthorchis varunai* was recorded from the gallbladder. The fish was collected from the Varuna River in Varanasi. The newly reported species has been described in detail, discussed and compared with other species of the genus *Opisthorchis*. It has elongated spinose body, bluntly pointed at anterior and posterior extremities, entire ovary, ventral sucker pre-equatorial, unlobed testes and presence of pre-pharynx. It has been distinguished by other species through the absence of a crown of spines around the excretory pore.

Keywords: Opisthorchiidae, Parasite, Trematoda, Varuna, *Wallago*.

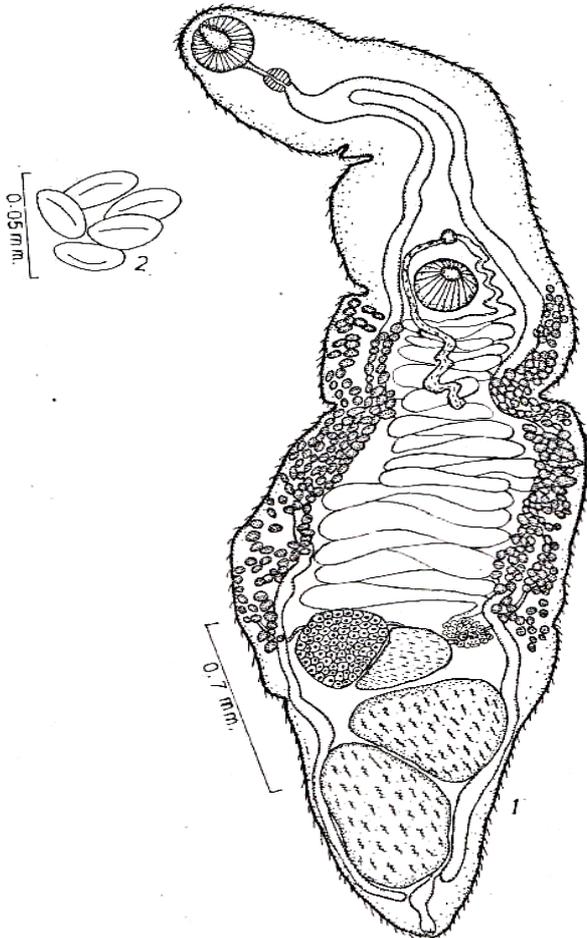
Introduction

Looss (1899) [8] erected the family Opisthorchiidae. Phylum Platyhelminthes includes animals with dorsoventrally flattened body hence the name flatworms (Verma and Prakash, 2020) [19]. This phylum is retained as such both in five and six kingdom systems (Verma, 2016a, 2016b) [15, 16] and is divided into three classes namely Turbellaria, Trematoda and Cestoda (Verma, 2017) [17]. The class Trematoda comprises a large number of families including family Opisthorchiidae. The family Opisthorchiidae comprises a large number of pathologically important genera. One important genus of this family is *Opisthorchis* that infects humans and other Piscivorous mammals like dogs, cats, pigs, house rats etc. *Opisthorchis* genus is liver fluke parasites that infect humans due to raw or uncooked fish intake. Blanchard (1895) [5] described the digenetic trematode genus *Opisthorchis*. *Opisthorchis* infection is worldwide problem, therefore, lot of works reported all around the world. Saenna *et al.* (2017) [13] reported Fish sharing risk factor for *Opisthorchis* infection in Thailand. Pentey *et al.* (2013) [11] described the zoonotic fish-borne liver flukes. Boonmars *et al.* (2009) [6] worked on Animal models for *Opisthorchis viverrini* infection. King and Scholz (2001) [7] observed trematodes of the family Opisthorchiidae. In India, several species of flatworms reported from genus *Opisthorchis*. Verma (1927) [18] described *Opisthorchis pedicellata* from *Bagarius yarrelli* and *Rita rita* from Allahabad. Rai (1971) [12] described *Opisthorchis gorakhpurensis* from *Mystus vittatus* from Gorakhpur. Agarwal and Singh (1978) [1] described *Opisthorchis thapari* from *Bagarius bagarius* from Lucknow. Pandey *et al.* (2004) [10] described *Opisthorchis attu* from *Wallago attu* from river Ganges at Varanasi. The present study therefore, focuses on the detailed description of the discovered parasite species and its comparison with already known species. The key of the genus *Opisthorchis* is also formed here.

Materials and methods

Fresh specimens of *Wallago attu* were collected from the river Varuna in Varanasi and transported to the laboratory of Zoology Department, DAV College, Kanpur (U.P.). For collection of digenetic trematodes visceral organs were dissected out separately in petri dishes containing 0.7% saline. The worm was picked up with the help of micro dropper and collected in saline, fixed in A.F.A. (50% alcohol, formalin and acetic acid in the ratio of 100:6:2.5) under slight pressure of coverslip for 24 hours. After fixation parasite was kept in A.F.A. for some time, washed in 70% alcohol to remove excess of fixative and finally preserved in 70% alcohol containing 5% glycerine. Specimen for whole mount was stained in acetic alum carmine, differentiated in acid water, dehydrated in graded series of alcohol, cleared in clove

oil and mounted in Canada balsam. The worm was also examined under microscope particularly for the excretory system. The diagram was made with the help of camera Lucida and the measurement was taken by using stage micrometre. Only one specimen of this form was collected from the gallbladder of a fresh water fish, *Wallago attu* (Bl.) at Varanasi. Its holotype is available at Department of Zoology, DAV College, Kanpur (U.P.), India.



Opisthorchis varunai sp. Nov.

Fig 1: Entire ventral view Fig 2. Eggs

Results and discussion

Body elongate, spinose with bluntly pointed anterior and posterior extremities, 4.15 mm long, 1.15 mm wide. Oral sucker sub-terminal, sub-spherical, 0.21 mm long, 0.22 mm wide. Pre-pharynx short, tubular 0.07 mm long. Pharynx is well developed, sub-globular 0.09 mm long, 0.08 mm wide. Oesophagus small, 0.13 mm long. Ventral sucker pre-equatorial, sub-spherical and slightly smaller than oral sucker, 0.20 mm long, 0.21 mm wide, sucker ratio 1.53 mm from anterior end of the body.

Excretory bladder tubular, sigmoid passed between testes (as observed in living specimen), excretory pore terminal.

Genital pore sub-median, inter-caecal, post bi-furcal just above the ventral sucker, at 1.37 mm from anterior end of the body.

Testes entire, unequal, separate, tandem, lying in posterior forth of body. Anterior testis 0.43 mm long, 0.53 mm wide at 3.1 mm from anterior extremity. Posterior testis larger than anterior testes, 0.60 mm long, 0.40 mm wide, at 0.22 mm from posterior extremity. Cirrus sac absent. Vesicula

seminalis long, tubular, serpentine and free in parenchyma, extending posteriorly up to 0.50 mm from posterior margin of ventral sucker. Ovary entire, sub-median, pre-testicular, post-equatorial, 0.31 mm long, 0.28 mm wide, at 2.74 mm from anterior end of body. Receptaculum seminis saccular, smaller than ovary, 0.32 mm long, 0.21 mm wide, lying between ovary and anterior testis. Vitellaria follicular, extending along caeca, commencing from posterior third level of ventral sucker up to mid-level of ovary. Ootype surrounded by Mehlis' gland cells present above receptaculum seminis. Uterus highly coiled, inter-caecal, extending between ovary and ventral sucker opening terminally into genital pore. Eggs numerous, oval, small, 0.27 to 0.32 mm long, 0.013 to 0.017 mm wide.

Host	:	<i>Wallago attu</i>
Location	:	Gall bladder
Locality	:	River Varuna, Varanasi
Prevalence	:	One specimen from 1 host, out of 4 examined.
Holotype	:	Available at Department of Zoology, DAV College, Kanpur

The present form is referred to the genus *Opisthorchis* (Blanchard, 1895)^[5] in the nature and commencement of vitellaria, presence of well-developed pre-pharynx and relative position of gonads. Yamaguti (1971)^[20] listed following species of this genus from Indian fresh water fishes viz., *O. pedicellata* (Verma, 1927)^[18], *O. mehrai* (Agarwal, 1959)^[2] and *O. thapari* (Mehra, 1941)^[9]. Later on *O. gorakhpurensis* (Rai, 1971)^[12], *O. gwaliorensis* (Bhadauria and Dondotia, 1977)^[3], *O. caudalspinatum* (Bhadauria and Dondotia, 1979)^[4] and *O. attui* (Pandey *et al.*, 2004)^[10] were added from fresh water fishes. Rai (1971)^[12] during studies observed some variations in his collections and on that basis synonymised *O. mehrai* and *O. thapari* with *O. pedicellata*. Author agrees with the above synonymy. In the opinion of author *O. thapari* shows more close resemblance with *Gomtia* (Thapar, 1930)^[14] in the nature and commencement of vitellaria, longer pre-pharynx in relation to oesophagus and in the relative position of gonads, hence, *O. thapari* is transferred to the genus *Gomtia* as *Gomtia (Opisthorchis) thapari* (Agarwal and Singh, 1978)^[11].

The new form resembles with *O. pedicellata*, *O. gorakhpurensis*, *O. gwaliorensis* and *O. caudal spinatum* in having entire ovary. However, it differs from *O. pedicellata* in having entire testes, smaller sucker ratio (1:0.9 instead of 1:1.5) and continuous vitellaria commencing from midlevel of ventral sucker, from *O. gorakhpurensis* in the presence of distinct pre-pharynx, extension of vitellaria and shape and position of receptaculum seminis, from *O. gwaliorensis* and *O. caudal spinatum* in having pre-equatorial ventral sucker and genital pore and in the more anterior commencement of vitellaria. It further differs from *O. caudal spinatum* in the presence of a crown of spines around the excretory pore. Accordingly, it is regarded as a new species with the specific name, *Opisthorchis varunai* sp. nov.

The new species is named after its locality from where the host was procured.

Key to the valid Indian freshwater species of the genus, *Opisthorchis* (Blanchard, 1895)^[5]:

- 1) Ovary entire-----2
Ovary notched-----*O.*

- attui* (Pandey *et al.*, 2004) ^[10]
- 2) Crown of spines present
around excretory pore-----*O.*
caudalspinatum (Bhadauria
and Dondotia, 1979) ^[4]
Crown of spines absent
around excretory pore----- 3
- 3) Ventral sucker pre-equatorial or equatorial----- 4
Ventral sucker distinctly post-equatorial-----*O.*
gwaliorensis (Bhadauria
and Dondotia, 1977) ^[3]
- 4) Testes lobed----- *O.*
pedicellata (Verma, 1927) ^[18]
Testes unlobed----- 5
- 5) Pre-pharynx present----- *O.*
varunai sp. Nov.
Pre-pharynx absent----- *O.*
gorakhpurensis (Rai, 1971) ^[12]

Conflict of interest

No conflict between authors.

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