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NEW LOCALITY RECORD OF *GENUS OPISTHORCHIS JONESAE* BILQEES AND KHAN, 2006 (TREMATODA: OPISTHORCHIIDAE) FROM THE LIVER OF *MILVUS MIGRANS* (BLACK KITE) IN LARKANA, SINDH, PAKISTAN

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Abstract

During the survey of helminth parasites of bird *Milvus migrans* (Black kite) Boddaert, 1783 of District Larkana, Sindh Pakistan, twelve (12) specimens were recovered from the liver of three (03) hosts. Present specimens are closer to the genus *Opisthorchis* Blanchard, 1895 in specific characters such as general body shape, position and shape of suckers, bulb shaped acetabulum, shape and size of ovary, anterior testis bi or tri-lobed, posterior testis penta-lobed, arrangement of vitelline follicles.

Keywords: *Opisthorchis*, Opisthorchiidae, *Milvus Migrans*, Larkana, Sindh, Pakistan.

INTRODUCTION

The genus *Opisthorchis* Blanchard, 1895 belongs to the family Opisthorchiidae Braun, 1901 and sub-family Opisthorchiinae Looss, 1899.

Available literature reveals several species of the genus *Opisthorchis,* reported from all over the globe Yamaguti, 1971 from fish, reptiles, birds and mammals.

Twenty-one species reported from Avian hosts include ten species from India, other species are from Russia, Turkestan, W. Siberia, Tuva, Europe, Hungary, Kirgiza, George SSR, China, Uzbekistan, Indochina, N. Queensland, Tashkent and Egypt (Yamaguti, 1971).

A single species reported from Pakistan is *O. lobatus* (Bilqees *et al*., 2003) later on Bilqees *et al.* (2003) created *Neometorchis lobatum* from liver of the duck. *Neometorchis* was synonmized with *Opisthorchis*.

Species reported from mammals are: *O. felineus* (Rivolta, 1884) Blanchard, 1895; *O. felinus arvicola* Sidorov, 1964; *O. felineus winogradovi* Goryachev, 1958; *O. starkovi* Biocca et Bennetti, 1956; *O. tenuicollis* (Rud., 1819) Stiles et Hassal, 1896; *O. tankae* Wallace et Panner, 1939; *O. viverrini* (Poirier, 1886) Stiles et Hassel, 1896; *O. wardi* (Wharton, 1921) Yamaguti, 1971.

Present specimens were recovered from *Milvus migrans*, Black Kite from Larkana, Sindh, Pakistan.

The black kite (*Milvus migrans*) is a Large-sized bird of prey in the family Accipitrida. Black kites (*Milvus migrans*) is found in Australian, Ethopian, Oriental and Paleacrtic regions. They live in different habitats. They mostly reside in open areas, wetlands, forests, towns, cities, deserts, agricultural lands etc. They feed on reptiles, insects, birds, fish, mammals etc. It is believed that these birds are mostly found in Asia and Africa.

MATERIALS AND METHODS

Four birds *Mivus migrans* (Black kite) were caught alive from District Larkana, Sindh at random intervals and brought to the parasitology laboratory, Department of Zoology, University of Sindh, Jamshoro, Pakistan. The birds were anesthetized, dissected and examined for collection of internal helminth parasites. During examination of gut contents and visceral organs twelve mature specimens were collected from the liver of three birds. Later these specimens were fixed in hot steaming 70% ethanol, where trematodes expand and instantly die. Later the specimens were gently placed over clean glass slide, pressed lightly with another, tied with thread and fixed in F.A.A. solution for twenty-four hours, stained with Mayer's carmalum, dehydrated in graded series of ethanol, cleared in clove oil and rinsed with xylene. Finally, the specimens were permanently mounted in Canada balsam for further study. Line Drawings were prepared with the aid of a camera Lucida. Measurements are in millimeters (mm) and length by width. Photomicrographs were prepared with the courtesy of Department of Zoology, University of Karachi.

RESULTS
(Figs: 1-2)

Host:	*Milvus Migrans* (Black kite)
Locality:	Larkana Sindh, Pakistan
Site of infection	Liver
Number of hosts examined/infected:	04/03
Number of specimens recovered:	12
Prevalence:	75%

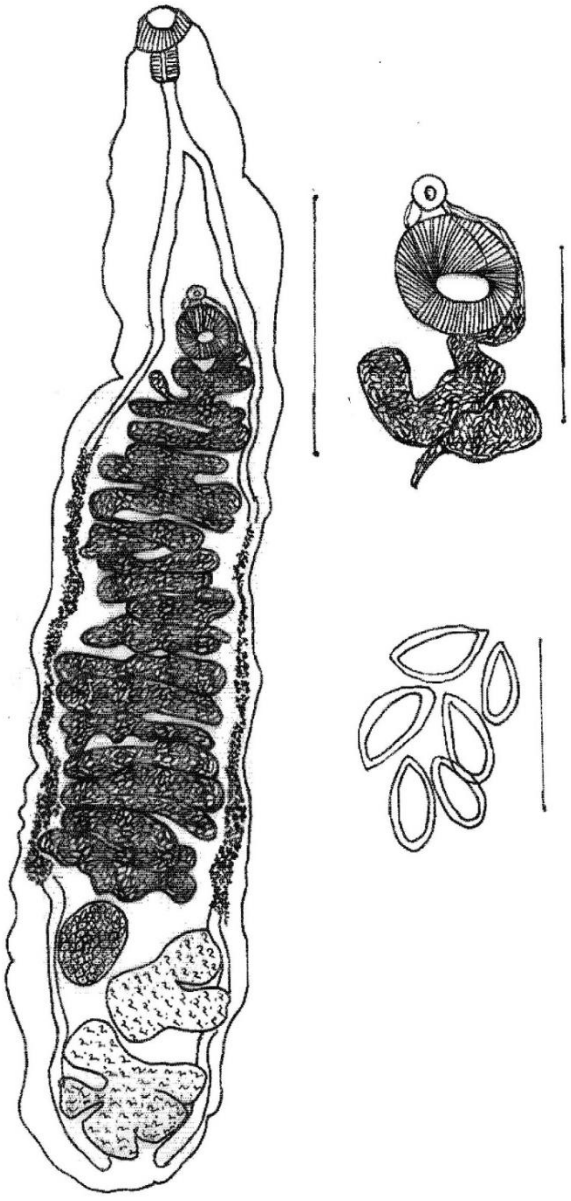

Fig. 1: *Opisthorchis jonesae* (Bilqees and Khan, 2006) Suleman *et al.*, 2016
a. *Opisthorchis jonesae*, entireworm
b. Acetabulum with cirrus sac and genital opening
c. Enlarged eggs

Fig. 2: *Opisthorchis jonesae* (Bilqees and Khan, 2006) Suleman *et al.*, 2016 Photomicrograph (5x10).

Table 1. Comparative body measurements of various species of genus *Opisthorchis* Blanchard, 1895 reported from avian hosts all over the globe.

S. No.	Species Name	HOST	LOCALITY	BODY SIZE	EGG SIZE
1	*O. jonesae* (present species)	*Milvus migrans*	Pakistan	3.94-4.75 by 0.80-0.99	25-27 by 22-24
2	*O. ahingii*	*Ahinga melanogaster*	India	8.7-16.5 by 0.42-0.95	23.1-26.4 by 12.5-13.02
3	*O. allahabadi*	*Sarcogyps calvus*;	India	5.5-6.5 by 2.01	23.1 by 13.2
4	*O. altaevi*	*Ardea purpurea*	Daghestan	5.0-7.0 by 0.63	25 by 12
5	*O. asiaticus*	*Aquila imperialis, Circus cinereus, C.aerugirosus*	Russian, Turkestan, W.Siberia, Tuva, A. Purpurea, Azerbaidzhan	13.5-14 by 0.21-0.34	26.1-29 by 14.5
6	*O. cheelis*	*Milvus migrans*	India	6.8 by 1.5	25 by 10
7	*O. choledochus*	*Anas*	`Europe	not given	not given
8	*O. dendriticus*	*Antigone antigone, Ardea purpurea*	India, Russia	8.2-15.5 by 0.65-1.3	not mention
9	*O. entzi*	*Ardea purpurea*	Hungary	12-13.2 by 1.0-1.2	24.2 by 13.2-15.4
10	*O.geminus*	*Milvus parasiticus, M.migrans, M.korschun, Anas boschas, Aquila clangula, A. Rapax, Circus aeruginosus, C. Melanoleucus, C.cyaneus, Ardea purpurea, Ardeola ralloides, Sarcogyps calvus*	Africa, Europe, Kirgizia, SSR, Sibria, India	7-12.5 by 1.3-2.0	21-27 by 10-13
11	*O. geminus falconis*	*Falco*	China	5.3 by 1.45	24-28 by 12.14
12	*O. geminus kirghisensis*	*Corvus aeruginosus, Buteo hurmanicus, Milvus linearis*	Kirgizia, Uzbekistan, Indochina	6.29-6.63 by 1.8	31.9 by 17.4
13	*O. giddhis*	*Sarcogyps calvus*	India	4.9 by 1.5	22.5 by 10
14	*O. indicus*	*Astur gentilis*	Allahabad	7 by 1.215	26.4 by 13.2
15	*O. milvusensis*	*Milvus govinda*	India	9 by 1.12	24-32 by15.5-16
16	*O. obsequens*	*Hieracidea berigora, H.orientalis, Netta rugina*	N. Queensl, W.siberia	2.6-5.1 by 0.8-1.2	25-30 by 14-17
17	*O. pelecani*	*Pelacanus,onocortalus*	India	5.0-6.5 by 0.75-0.8	23-26.4 by 16.5
18	*O.schikhobalovi*	*Fulica atra atra*	Tashkent, Oblast, Uzbekistan	6.0-6.8 by 2.08	Not mention
19	*O. simulans*	*Pernis apivoris, Aythya, Anas, Nyroca, Anser, Circus aeruginosus, Haliaetus, Fuligula*	Egypt, Europe, Siberia	7-23 by 1.0-1.5	28-29 by 16-8
20	*O. tenuicollis geminus*	*Milvus parasiticus*	India	6-11.5 by 1.6-2 .355	26.4 by 13.2
21	*O. tsingkianpuensis*	Domestic fowl	Kiangsu, China	9.8-13.8 by 1.12-1.55	23-26 by 10-14
22	*O. vitellatus*	*Phalacrocorax auritus*	Illinois River	3.45-4.03 by 0.43-0.53	25 by 13
23	*O. lobatus*	Duck	Pakistan	not mention	not mention
24	*O. longissimus*			not mention	not mention

DESCRIPTION IS BASED UPON TWELVE MATURE, EGG BEARING AND PERMANENTLY MOUNTED SPECIMENS:

Body flattened, elongate, anterior end narrower, with posterior end rounded, slightly curved below the acetabulum, measure 3.94-4.75 (4.35). Maximum width attained at the level of gonads 0.80-0.99 (0.90).

Oral sucker terminal, rounded, wider than long measure 0.07-0.16 (0.12) by 0.10-0.24 (0.15). Pre-pharynx absent, pharynx rounded to elongated, highly muscular measure 0.07-0.13 (0.11) by 0.06-0.10 (0.09).

Esophagus long 0.07-0.29 (0.17) bifurcate at some distance below the pharynx, divides into two long intestinal caeca terminating posterior to testes. Distance between caecal bifurcation and acetabulum is 0.54.

Acetabulum roughly bulb shaped, narrower at anterior end and wider at base, located at 2nd quarter of the body measure 0.17-0.26 (0.20) by 0.17-0.21 (0.19). Distance between oral sucker and ventral sucker is 1 mm.

Ovary oval to rounded, situated at 4th quarter of the body above the anterior testis measure 0.14 -0.41 (0.30) by 0.17-0.23 (0.20).

Testes tandem, multilobed, totally inter-caecal, located at posterior region of the hind body. Anterior testis bi or tri-lobed measure 0.32-0.54 (0.40) by 0.33-0.51 (0.42) and posterior testis penta-lobed, slightly larger than anterior testis measure 0.38-0.54 (0.48) by 0.35-0.60 (0.51). Cirrus pouch rounded just above the acetabulum, genital pore preacetabular below the intestinal bifurcation.

Vitelline follicles commence from some distance below the acetabulum and proceed up to the level of ovary in the mid of hind body.

Uterus with numerous coils, start from pre-ovarian region and extend up to the level in posterior of acetabulum.

Eggs oval to elongated, double shelled, thin-walled measure 0.05-0.07 (0.06) by 0.02-0.04 (0.03).

DISCUSSION

Available literature reveals several species of the genus *Opisthorchis*, are reported from all over the globe (Yamaguti, 1971). Species are reported from fish, reptiles, birds and mammals. Yamaguti, 1971 reported 21 species of genus *Opisthorchis* from avian hosts, of which species recovered from India are*: O. ahingii* (Mehra, 1941) found in Ahinga melanogaster; *O. allahabadi* (Mehra, 1941) found in *Sarcogyps calvus*; *O. cheelis* (Lal, 1939) in *Milvus migrans* from Lucknow; *O. dendriticus* (Morgan, 1927) in *Antigone antigone; O. geminus* (Looss, 1896) Looss, 1899 reported in *Milvus parasiticus, M. migrans* etc. This species are also reported in Africa, Europe, Kirgizia, George SSR, Siberia. *O. giddhis* (Lal, 1939) reported in *Sarcogyps calvus* from Lucknow; *O. indicus* (Mehra, 1941) found in *Astur gentilis* from Allahabad; *O. milvusensis* (Murhar, 1959) in *Milvus govinda; O. pelecani* (Mehra, 1941) in *Pelacanus, onocortalus* and *O. tenuicollis geminus* (Looss, 1896) Erhardt, 1935 in *Milvus parasiticus*.

Body size in present specimens is (3.94-4.75 by 0.80-0.99) and differs from the below mentioned avian Species in having smaller body size *O. ahingii* (8.7-16.5 by 0.42-0.95); *O. allahabadi* (5.5-6.5 by 2.01); *O. altaevi* Saidov, 1954 (5.0-7.0 by 0.63); *O. asiaticus* Skrjabin, 1913 (13.5-14 by 0.21-0.34); *O. cheelis* (6.8 by 1.5); *O. dendriticus* Morgan, 1927 (8.2-15.5 by 0.65-1.3); *O. entzi* Ratz, 1900 (12-13.2 by 1.0-1.2); *O. geminus* (7-12.5 by 1.3-2.0); *O .geminus falconis* Tang, 1941 (5.3 by 1.45); *O. geminus kirghisensis* Skrjabin, 1913 (6.29-6.63 by 1.8); *O. giddhis* (4.9 by 1.5); *O. indicus* Mehra, 1941 (7 by 1.215); *O. milvusensis* (9 by 1.12); *O. pelecani* Mehra, 1941 (5.0-6.5 by 0.75-0.8); *O. schikhobalovi* Sultanov, 1962 (6.0-6.8 by 2.08); *O. simulans* Looss, 1896 (7-23 by 1.0-1.5); *O. tenuicollis geminus* Looss, 1896 (6-11.5 by 1.6-2 .355); *O. tsingkianpuensis* Hsu et chow, 1938 (9.8-13.8 by 1.12-1.55).

Body size of *O. giddhis* is 4.9 by 1.5; *O. vitellatus* (Chin, 1950) 3.45-4.03 by 0.43-0.53 and *O. obsequens* (Nicoll, 1914) 2.6-5.1 by 0.8-1 having approximately same size and body size of *O. lobatus, O. longissimus* (Bisseru, 1957) is not mentioned in the literature.

In present specimens the oral sucker is terminal, while in *O. simulans* it is sub-terminal. In *O. longissimus* and *O. lobatus* oral sucker is also terminal.

In present specimens and in *O. lobatus, O. longissimus* the pharynx is oval to elongated, in *O. simulans* it is globular in shape.

The present specimens have acetabulum roughly bulb shaped, while in *O. simulans, O. lobatus* and in *O. longissimus* it is rounded in shape.

Ovary in present specimens is rounded in shape, while in *O. simulans* it is bilobed, in *O. longissimus* it is oval and overlapped by anterior testis and in *O. lobatus* it is irregular in shape (asymmetrical).

In present species the anterior testis is bi or trilobed, sub-median, approximately at the lateral side of the body and posterior testis is penta lobed, while in *O. simulans* both testes are median, the anterior testis is rounded, horizontally elongated and posterior testis is roughly oval and vertically elongated; in *O. longissimus* anterior testis is tetra lobed and posterior testis is tri-lobed and in *O. lobatus* both testes are median, anterior testis is penta lobed and posterior testis is hexalobed.

In present specimens vitelline follicles start at a little distance from the acetabulum above the mid region of the body and extend up to the level of the ovary, while in *O. simulans* vitelline follicle commence from acetabulum, approximately below the middle of the body and proceed upto the level of anterior testis, in *O. longissimus* the vitelline follicles start from middle of the body and extend up to above the ovary and in *O. lobatus* the arrangement of vitelline follicle is same as in present specimens.

Eggs size in present specimens is 25-27 by 22-24 and it differ from *O. ahingii* 23.1-26.4 by 12.5-13.02; *O. allahabadi* 23.1 by 13.2; *O. altaevi* 25 by 12; *O. asiaticus* 26.1-29 by 14.5; *O. cheelis* 25 by 10; *O. choledochus* (not given); *O. dendriticus* (not mention); *O. entzi* 24.2 by 13.2-15.4; *O. geminus* 21-27 by 10-13; *O. geminus falconis* 24-28 by 12.14; *O. geminus kirghisensis* 31.9 by 17.4; *O. giddhis* 22.5 by 10; *O. indicus* 26.4 by 13.2; *O. milvusensis* 24-32 by 15.5-16; *O. obsequens* 25-30 by 14-17*; O. pelecani* 23-26.4 by 16.5; *O. schikhobalovi* (not mention); *O. simulans* 28-29 by 16-8; *O. tenuicollis geminus* 26.4 by 13.2*; O. tsingkianpuensis* 23-26 by 10-14; *O. vitellatus* 25 by 13 and egg size of *O. lobatus, O. longissimus* is not mentioned in the literature.

Present specimens closely resembles with *O. jonesae* (Bilqees and Khan, 2006) Suleman *et al.,* 2016 in general body shape, shape and size of suckers, shape, size and position of gonads, shape and size of eggs and distribution of eggs. Originally the species was recorded from small intestine of *Milvus migrans* from Swabi, the present specimens are reported from the same avian host *Milvus migrans* while the site of infection was liver and locality Larkana, Sindh Pakistan. The locality is new and first record for the species.

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