

## ***Neodiplostomum karachiensis*, New Species (Trematoda: Diplostomoidea, Poirier, 1886) From Black Kite *Milvus migrans* in Karachi, Sindh, Pakistan**

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**Abstract.-** A new species *Neodiplostomum karachiensis* collected from the small intestine of black kite *Milvus migrans* in Karachi, Sindh, Pakistan is being described. It is characterized by an elongated fore-body with broader, posterior end, cup-shaped hind-body with somewhat pointed posterior end. The fore-body has lateral margins curved ventrally ventral sucker prominent, above the hold-fast organ which is situated in the posterior region of the fore-body, uniform distribution of vitelline follicles in the fore-body, bursa copulatrix sub-terminal in position.

**Key words:** *Neodiplostomum karachiensis*, new species, Diplostomoidea, *Milvus migrans*, Digenea.

### INTRODUCTION

*Neodiplostomum* Railliet, 1919; Syns. *Conchogaster* Lutz, 1928; *Triplostomum* Lutz, 1928; *Neodiplostomoides* Vidyarthi, 1938 is a well established genus with numerous species reported from almost all over the world from avian hosts.

The *Neodiplostomids* belong to the super family *Diplostomoidea* Poirier, 1886 which are distinctly different from other groups of trematodes in possessing a unique cup- shaped hind body, hold fast organ, situated a little above the end of fore body, where the specimen attains maximum width of the fore body. The hold fast organ plays both adhesive and digestive roles and a bi-partite body.

Yamaguti, 1958; 1971 have listed about 76 species of the genus *Neodiplostomum* from a variety of avian hosts. Additionally several species have been reported from Indian birds. On the contrary, *N. spathoides* Dubois, 1937, Syns. *Diplostomum* (*Neodiplostomum*) *spathula* Brandes, 1888; *N. palumbarii* Dubois, 1937; has been reported from *Milvus migrans* in Pakistan (Bhutta and Khan, 1975). Present specimen has been recovered from the same host, which appears to be new to science, therefore it is described and reported from

Sindh, Pakistan, reported from Sindh, Pakistan, and proposed to be a new species *Neodiplostomum karachiensis*. Species name refers to the locality of the host bird.

### MATERIALS AND METHODS

Five Black Kites *Milvus migrans migrans* (Boddaert) were purchased from a local bird market in Karachi at random intervals. The birds were autopsied in the laboratory for collection of internal helminth parasites. One out of five birds was found infected with a minute trematode. The worm was thoroughly washed in the saline solution, and left in distilled water for few minutes. Later it was fixed in hot alcohol-formalin acetic acid solution. The trematode was gently placed over a clean glass slide, with slight pressure with another, tied with thread and placed in F.A.A. solution for twenty four hours. The specimen was washed with 70% ethanol, stained with Mayer's Carmalum, dehydrated in graded alcohol, cleared in clove oil, rinsed with xylene and mounted permanently in Canada balsam. Drawings were made with the aid of a Camera Lucida, measurements are length by width, in millimeters. Photomicrograph was prepared through Olympus Digital Microscope MIC-D. Measurements are in Millimeters.

Holotype specimen is deposited in the senior author's collection, Department of Zoology University of Karachi, Karachi 75270.

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***Neodiplostomum karachiensis*, new species****(Fig. 1)**

Host	<i>Milvus migrans</i> (common black kite)
Locality	Karachi city
Location	Small intestine
No. of hosts examined/infected	5/1
No. of specimen recovered	01

Description is based upon a single permanently mounted mature specimen: Body length 1.85; distinctly subdivided by a constriction into fore and hind body parts, being wider at the level below the hold fast organ in the fore body 0.65 and 0.75 in the hind body region at the level below the ovary. Fore body 1.10 by 0.65 at its greatest width, elongated with lateral edges curled ventrally. Hind body 0.75 by 0.75; cup shaped, broader in anterior mid- region and pointed in the posterior region, smaller in length than the fore body.

Suckers well developed; oral sucker terminal, rounded 0.060 by 0.072. Acetabulum larger than the oral sucker, in front of middle of the fore body 0.09 by 0.09. Suckers ratio is: 1:1.85. Hold fast organ 0.28 by 0.17, oval, elongated, situated in the posterior region of the fore body.

Pharynx about the size of the oral sucker 0.06 by 0.057, elongated, prepharynx absent. Esophagus small, clearly visible 0.050 long. Intestinal ceca thin pass besides the ventral sucker and the hold fast organ.

Testes tandem, dissimilar, anterior testis asymmetrical 0.21 by 0.41, situated in the anterior right corner of the hind body. Posterior testis bilobed, concave ventrally with a slight median incision, the right lobe is 0.20 and the left lobe is 0.2 long. Bursa copulatrix not clearly visible however light impression appears in the posterior end of the hind body measuring 0.10 by 0.10.

Vitelline follicles profusely and uniformly distributed in the fore body, well above the ventral sucker reaching a little distance behind the cecal bifurcation. The vitellaria extends up to the posterior body region in the hind body. The vitelline follicles are nearly absent in the lateral margins of the hind body. In the hinder most region of the body the vitelline follicles appear to aggregate below the posterior testis.



Fig. 1. *Neodiplostomum karachiensis*, new species; A, entire specimen; B, enlarged view of Posterior region with genital opening.

Ovary oval to rounded, situated in the anterior most region of the hind body, touching the border line of the fore body 0.22 by 0.29.

**DISCUSSION**

There is a single species of the genus *N. spathoides* Dubois, 1937. Syns. *Diplostomum spathula* Brandes, 1888, *Neodiplostomum palumbarii* Dubois, 1937, *Neodiplostomum (Neodiplostomum) prudhoei* Bisseru, 1956, reported from Pakistan, from the common black kite, *Milvus migrans* Bhutta and Khan, 1975. Present species is a second record of the genus reported from the same

host i.e. the common black kite *Milvus migrans* in Sindh, Pakistan.

A search record of species of the genus from *Milvus* sp. From British Museum (N. H.) is as under:

*N. spathula* Creplin, 1829 from *Milvus korschun* in the wild locality in European USSR; *N. pseudattenuatum* Dubois, 1928 from *Milvus migrans* and *M. korschun* in the wild locality in Japan; and USSR respectively; *N. obscurum* Dubois, 1937 from *Milvus migrans* in the wild locality in Hungary; *N. perlatum* Ciurea, 1911 from *Milvus korschun* in the wild locality in the USSR; *N. spathoides* Dubois, 1937 from *Milvus korschun* in the wild locality in USSR.

The present specimen is distinguished from *N. spathula* (Crepl., 1829) La Rue, 1926 in the shape, size and structure of the fore and hind body, shape, size and position of the holdfast organ, size and position of the ovary and shape, size and position of the testes. The anterior testis in present specimen is asymmetrical and the posterior testis is symmetrical, while in *N. spathula* the testes are dumb-bell shaped nearly reaching the lateral margins of the hind body, Ovary in the present specimen is oval to rounded in shape, while in *N. spathula* it is globular to rounded in shape, in present specimen the ovary is larger in size as compared to that in *N. spathula*, distribution pattern of vitellaria is different in the present specimen (Fig. 1). A different host *Accipiter nisus* and different locality serve present specimen different from *N. spathula*.

*N. gavalis* Narain, 1930 was described from the intestine of *Gavialis gangeticus* in Allahabad, India. Dubois, 1938 preferred to retain its original nomenclature owing to insufficiently described genital system and assigned it to the genus *Crocodilicola*.

The present form can also be distinguished from other reported species mainly in the shape, size and structure of the fore and hind body. The present specimen appears totally different from *N. pricei* Krull, 1934 reported from *Larus novaehollandiae* from USA, mainly in having an elongated fore body with its posterior region broader than the anterior, fore body longer than the hind body. The hind body is cup-shaped. Oral sucker is spherical and nearly

equal to the pharynx, fairly small esophagus. The body is devoid of fine spines as stated to be present in the anterior body region in *N. pricei*. The hold fast organ in present specimen is oval and elongated situated at the posterior border of fore body, with median longitudinal cleft. The anterior testis in present form is asymmetrical while the posterior testis is symmetrical, the anterior testis is oval and globular in shape situated in the anterior left portion of the hind body while the posterior testis is obliquely placed and it is somewhat dumb-bell shaped with its lateral lobes nearly reaching the lateral margins of the hind body. Ovary in the present form lies in the hind body while in *N. Pricei* it lies posterior and lateral to anterior testis. Distribution of vitellaria is different in the present specimen additionally a different host *Milvus migrans* and a different locality serves present form to be different from *N. pricei*.

*N. tytense* Patwardhan 1935 from the intestine of *Tyto alba stertens* in Nagpur, India, was again recorded by Verma (1936a) from the Osprey and the common vulture in Allahabad. Verma (1936a) reported from a Kestrel in Allahabad somewhat similar forms but he reserves his final opinion that they may be different species.

*N. cuckooi* Verma 1936a originally described this species from a Cuckoo in Allahabad and assigned it to a newly created genus *Procrassiphiala* but as the host is of different order and as the posterior portion is smaller than the anterior Dubois (1938) placed it in the genus *Neodiplostomum*.

There are several species reported from Indian subcontinent viz.,

*N. globiferum* Verma 1936a from the intestine of a Cuckoo in Allahabad.

*N. laruei* Vidyarthi 1938a was described from the intestine of *Sarcogyps calvus* and *N. mehranium* Vidyarthi 1938a from the intestine of *Haliaetus leucoryphs*.

The terminal genitalia and bursa copulatrix are said to be improperly described in all the above mentioned three species (Bhalerao, 1942).

Vidyarthi 1938a described *Neodiplostomoides mehrii* from the intestine of a Bonell's Eagle in Allahabad. Dubois 1938 assigned it to the well-established genus *Neodiplostomum* on

the basis of doubtful characters of the genital bulb, shape of testes etc.

Vidyarthi 1938b described *N. orientalis* from the intestine of *Buteo rufinus rufinus* in Allahabad; he assigned it to the genus *Bolbophorus*. But Dubois (1938) taking into account the host, pointed cephalic extremity and the structure of bursa copulatrix, considered the genus to be doubtful and regarded it to be a member of the genus *Neodiplostomum*.

Lal 1939 described *N. bagulum* from the intestine of the Eastern Grey Heron in Lucknow and assigned this species to the genus *Pharyngostomum* of the subfamily Alariinae. Bhalerao (1942) pointed out that members of the subfamily are parasites of mammals and the vitelline follicles are confined to anterior segment of the body only. This species having avian host and vitelline follicles being distributed in both fore and hind body can be assigned to the subfamily Diplostominae and to the genus *Neodiplostomum* on the basis of further generic characters.

Chatterji 1942 described *N. brachypteris* from the small intestine of three woodpeckers *Brachypternus bengalensis bengalensis* caught from Sulemsarai, a village near Allahabad. Present specimen differ from this species mainly in the body shape, size and structure of fore and hind body; different pattern of distribution of vitelline follicles, size of the oral and ventral suckers which are almost equal in *N. brachypteris*; shape, size and position of the hold fast organ and shape, size and position of the ovary and shape, size and position of the anterior and posterior testes and a different host and locality serve to distinguish present specimen from *N. brachypteris*.

*N. hawkei* Chatterji 1942 from the small intestine of Indian hawk *Accipiter nisus malanoschilus* caught from a village near Allahabad differs from the present specimen mainly in its body shape and size and structure of fore and hind body, size and position of hold fast organ, distribution of vitelline glands; position, size and shape of ovary; shape, size and position of testes and a different host and locality.

*N. nisus* Chatterji, 1942 from the Indian hawk *Accipiter nisus malanoschilus* is different from the present specimen in the shape, size and structure of the fore and hind body, distribution of the

vitelline follicles, nearly equal size of oral and ventral suckers, dissimilar shape, size and position of hold fast organ, shape, size and position of the ovary, asymmetrical shape of anterior testis, while posterior testis is symmetrical and a different host and locality.

Caballero 1944 reported *N. paraspathula* Noble, 1936 from the small intestine of *Aquila chrysaetos* in Mexico. Present specimen differs from this species in the fore and hind body shape and size, arrangement of vitelline glands; shape, size of hold fast organ is also different from the present specimen; shape, size and position of ovary; shape, size and position of the asymmetrical anterior testis, and a different host and locality.

*N. banghami* Penrod, 1947 from the intestine of an immature bald Eagle *Haliaeetus leucocephalus* Linne, 1766 in Ohio is distinguished from the present specimen in general body shape, size including structure of fore and hind body, smaller oral and ventral suckers, shape, size and position of hold fast organ, distribution of vitelline follicles in both fore and hind body regions; position and size of ovary; shape, size and position of the anterior and posterior testes and a different host and locality, serve to separate present form from *N. banghami*.

*N. buteonis* Dubois and Rausch, 1950 was described from the intestine of *Buteo jamaicensis borealis* (Gmelin). In *N. buteonis* the fore and hind body are nearly equal in size, it further differ from the present form in the distribution of vitelline follicles in the fore body; shape, size and position of ovary in the hind body; shape, size and position of the anterior and posterior testes; somewhat rounded shape of the hold fast organ and a different host and locality.

*N. attenuatum* (Linstow, 1906; La Rue, 1926) Dubois, 1970a was recovered originally from: *Milvus korschun* in the wild locality in Ukraine and Moldavia, later in *Buteo buteo* in Europe, also in *Strix aluco* and *Accipiter nisus*. In present specimen the fore body is elongated and hind body is cup shaped, the fore body is larger than the hind body, where as in *N. attenuatum* size of the fore and hind body are nearly equal. *N. attenuatum* differ from the present specimen in shape and size of the holdfast organ, size of the oral sucker and pharynx; shape,

size and position of the ovary, and shape, size of the anterior and posterior testes.

*N. (N.) spathoides* Dubois, 1937 have also been recorded and described from *Milvus migrans* in Lahore and two specimens of *Accipiter badius* from Gujranwala and Lahore, Punjab, Pakistan (Bhutta and Khan, 1975). The present species although described from the small intestine of the same avian host i.e. *Milvus migrans* in Sindh, Pakistan, is distinguished in the body shape and size, position, shape, size of the testes, and position and size of the ovary.

The present specimen also differs from *N. bilqeesae* sp.n. and *N. dharmaii* sp.n. (submitted for publication) recovered from the same host i.e. *Milvus migrans* mainly in the shape, size of the fore and hind body, sizes of the oral and ventral suckers, size of the pharynx and length of the esophagus. The vitellarium is profusely distributed in the  $\frac{3}{4}$ <sup>th</sup> portion of the fore body, well above the ventral sucker in present form (Fig.1).

Present form also differ from *N. kanpurensis* Gupta, 1980 reported from *Astur badius* in kanpur, India in having smaller body size with different shape of fore and hind body. In *N. kanpurensis* the testes are transversely elongated, vitelline follicles extend upto ventral sucker only. While in present form the vitelline follicles extend some distance below oral sucker, a different host and locality serves *N. kanpurensis* different from present form.

Chal and Shin, 2002 described *N. leei* on the basis of adult flukes recovered from the small intestine of chicks, experimentally infected with small sized *Neodiplostomula* from the grass snake *Rhabdophis tigrina*. It differs from present specimen in having extensive distribution of vitellaria, bilobed testes and smaller size of tribocytic organ.

The present form also does not match with *N. travassosi* (Dubois, 1937) Lunaschi and Drago, 2005 collected from the intestine of *Polyborus plancus* (Miller) and *Phalacrocorax brasilianus* (Gmelin) in Argentina. Originally this species was reported in *Scops cristatus*, *Syrnium perspicillatum* and *strix* sp. in Brazil. Body length of the both originally reported and later redescribed species are less than the present species: 1.7-1.72; 1.40-1.69;1.43. The body length in present form is 1.85. Present form is totally different in body shape both

fore and hind body. The tribocytic organ in present form is also larger in size being 0.28 by 0.17 while it is 0.18;0.14 and 0.14 in the redescribed species. The oral sucker in present form is smaller than the ventral sucker while it is larger than the ventral sucker in the redescribed species. Similarly the esophagus is moderately long in present form being 0.05, while it is 0.016-0.053; 0.035; 0.04-0.072 in the reported species. The size of the gonads are similarly smaller than the present form in the redescribed species. Additionally a new host *Milvus migrans* and locality i.e. Karachi, Sindh, Pakistan serve to differentiate the present specimen from *N. travassosi* (Dubois, 1937) Lunaschi and Drago, 2005. The worm at disposal of the authors is therefore described as a new species *N. karachiensis* n.sp.

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