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NEW HOST AND LOCLAITY RECORD OF *KNIPOWITSCHIATREMA STERNULAI* BUSHRA *ET AL*., 2016 (TREMATODA: HETEROPHYIDAE LEIPER, 1909) FROM THE SMALL INTESTINE OF *STERNULA SAUNDERSI* (BLACK-SHAFTED TERN) LARKANA, SINDH, PAKISTAN

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ABSTRACT

A study of the helminths in *Sternula Saundersi* (Black-shafted tern) was conducted in Larkana, Sindh, Pakistan. In total, Seventeen (17) trematodes were found from the small intestine. Genus *Knipowitschiatrema* Issaitschikow, 1927 includes only four number of species; however, the present specimens have all essential characteristics of *Knipowitschiatrema sternulai*, Bushra *et al*., 2016, therefore, present specimens are recognized as such. In contrast to *Knopowitschiatrema sternulai*, Bushra *et al.*, 2016, which was previously isolated from the small intestinal of *Sternula albifrons* (Little tern) in Hyderabad Sindh, the specimens in this study were collected from *Sternula Saundersi* in Larkana, Sindh, Pakistan. *Knipowitschiatrema sternulai* Bushra *et al*., 2016, is second time reported from Sindh in new host *Sternula Saundersi* and for the first time from new locality Larkana, Sindh, Pakistan.

Key Words: *Knipowitschiatrema sternulai*, *Sternula Saundersi*, Heterophyidae, Larkana, Sindh, Pakistan.

INTRODUCTION

The genus *Knipowitschiatrema* was created by Issaitschikow, 1927, belongs to family Heterophyidae Leiper, 1909 and sub-family Galactosominae Ciurea, 1933. Species of the genus have been reported from Black sea, South-Western Mediterranean, Alboran sea, France, Britain and Pakistan. The genus comprises only four species and two of them are reported from Hyderabad, Pakistan. Present specimens are similar to *Knipowitschiatrema sternulai*, Bushra *et al*., 2016 reported from *Sternula albifrons* in Hyderabad, Sindh, Pakistan in shape of body, size, shape and position of suckers, gonads, pre-pharynx, seminal vesicle and position of vitelline follicles therefore regarded as same. This is second report of *Knipowitschiatrema sternulai* Bushra *et al*., 2016 from new host *Sternula Saundersi* and locality Larkana, Sindh, Pakistan.

MATERIALS AND METHODS

Twelve *Sternula Saundersi* (Black-shafted tern) were purchased and trapped from different tehsils of District Larkana for examination of helminths infection. Garcia and Ash, 1979 and Schmidt, 1988, methods ware adopted for preparation of trematodes. Birds were anesthesized in sealed glass jar using chloroform and then dissected for examination of body cavity and visceral organs for collection of helminths. Seventeen trematodes belonging to the genus *Knipowitschiatrema*, Issaitschikow, 1927 were found in the small intestine of five hosts. Collected trematodes were fixed in Formalin Acetic Acid solution under slight pressure of cover glass slip for a day, dehydrated in 70%, 90%, 100% alcohol series, stained in Mayer's Carmalum and permanently mounted with Canada balsam. All measurements were noted in length by width in millimeters (mm). Line drawings were prepared with help of a Camera lucida attached with compound microscope. The genus was identified using the descriptions and keys given by Yamaguti (1971), McDonald (1981) and Gibson *et al.*, (2001). Specimens are in possession of Prof. Dr. Sanjota Nirmal Das, Sectional Head Parasitology, Department of Zoology, University of Sindh, Jamshoro.

RESULTS

KNIPOWITSCHIATREMA STERNULAI (Bushra *et al*., 2016)
(Fig. 1. A-C)

Host:	*Sternula Saundersi* Black shafted-tern
Locality:	Larkana, Sindh
Location:	Small Intestine
Number of hosts examined/ infected:	12/05

Number of specimens recovered: 17
Prevalence: 41.66%

DESCRIPTION IS BASED ON SEVENTEEN MATURE, EGG BEARING STAINED AND PERMANENTLY MOUNTED SPECIMENS:

Body small and sub cylindrical, with rounded ends, anterior end of body is more rounded than posterior end. Maximum width of body is attained behind the testes in the posterior region and measures 1.71-2.31 (1.92) by 0.53-0.67 (0.61) in diameter.

The oral sucker terminal, Smaller in size as compared to ventral sucker, wider than long and measures 0.13-0.17 (0.134) by 0.23-0.29 (0.249).

Prepharynx very small and measures 0.013-0.037 (0.021) by 0.026-0.033 (0.028) in size.

Pharynx muscular, `oval to spherical in shape and measures 0.038-0.042 (0.039) by 0.05-0.08 (0.06) in size.

Esophagus absent. Caeca starts above the acetabulum extend in lateral fields and terminate at the posterior end of anterior testis.

Ventral sucker is spherical in shape, present in the first quarter of body and larger in length and smaller in width than oral sucker and measures 0.21-0.27 (0.231) by 0.20-0.25 (0.211). Suckers ratio is 1:1.7. Distance between oral sucker and ventral sucker is 0.048-0.09 (0.065).

Seminal vesicle elongated, median, sac like with narrow anterior end and located on the right side of ovary and measures 0.26-0.30 (0.252) by 0.08-0.11 (0.087) in size. The distance between acetabulum and seminal vesicle is 0.4 (Fig.1, Table 1).

Table 1. Comparison between different species of the genus *Knipowitschiaterma* Issaitschikow, 1927.

Species	**Present specimens**	***K. nicolai*** Issaitschiko, 1927	***K. echinatum*** Timon David, 1955	***K. pakistanensis*** Bushra *et al*.,2015	***K. sternulai*** Bushra *et al*.,2016
Host	*Sternula Saundersi*	*Larus argentatus*	*Larus argentatus michallis; L. fuscus*	*Sternula albifrons*	*Sternula albifrons*
Locality	Larkana, Sindh	Russia	Britain, France	Hyderabad, Sindh	Hyderabad, Sindh
Body size	1.71-2.31 by 0.53-0.67	3.126-3.54 by 0.473-0.524	4.6-7 by 0.49- 0.69	2.89-3.25 by 0.36-0.4	1.66-2.27 by 0.51-0.64
Oral Sucker	0.13-0.17 by 0.23-0.29 terminal, wider than long and smaller than acetabulum	Sub-terminal, larger than the acetabulum	Not mention	0.13-0.16 by 0.17-0.19 terminal, smaller than acetabulum, more elongated.	0.11-0.15 by 0.22-0.27 terminal, smaller than the acetabulum, wider than long
Pre-pharynx	0.013-0.037 by 0.026-0.033, very small	Long and wide	Not mention	0.09-0.1 by 0.02-0.03 long.	0.01-0.03 by 0.023-0.03, short.
Pharynx	0.038-0.042 by 0.05-0.08, muscular and oval to spherical	Muscular, rounded	Not mention	0.11-0.11 by 0.13-0.13, globular.	0.034-0.04 by 0.04-0.07 rounded.
Esophagus	Absent	Absent	Not mention	0.49-0.51 by 0.09-0.09	Absent
Acetabulum	0.21-0.27 by 0.20-0.25, much larger than oral sucker and	Smaller than oral sucker, enclosed in genital atrium.	Not mention	0.26-0.27 by 0.23-0.23, larger than oral sucker, not enclosed in genital	0.19-0.24 by 0.18-0.23 larger than oral sucker, not enclosed in

	rounded to spherical			atrium.	genital atrium.
Distance b/w oral sucker & acetabulum	0.048-0.09	Not mention	Not mention	0.71-0.75	0.046-0.08
Seminal vesicle	0.26-0.30 by 0.08-0.11 , elongated, median, sac like	Quite larger, twisted in shape, Situated immediately below the acetabulum.	Not mention	0.42-0.47 by 0.11-0.13 larger, flask shaped, voluminous size, Situated immediately	0.23-0.27 by 0.07-0.09 smaller size, elongated, saccular with narrow anterior portion, Situated far below the acetabulum
Distance b/w seminal vesicle &acetabulum	0.4	Not mention	Not mention	Not mention	0.3
Ovary	0.15-0.19 by 0.13-0.15, oval, small, more towards the left side of body	Rounded, located near middle of the body.	Not mention	0.09-0.09 by 0.1-0.1 rounded, located near the posterior region of the body.	0.13-0.16 by 0.1-0.13 roughly spherical, located near middle of the body.
Testes	Ant: 0.095-0.14 by 0.19-0.25, oval to spherical and smaller Post: 0.18-0.24 by 0.21-0.25, oval and larger than anaterior testis	Rounded, equal in size	Not mention	Ant: 0.1-0.11 by 0.12-0.15, roughly spherical, smaller size Post: 0.14-0.18 by 0.15-0.16, oval, larger size.	Ant: 0.092-0.12 by 0.17-0.22 roughly rounded, smaller size Post: 0.16-0.2 by 0.18-0.22 rounded, slightly larger in size.
Uterus	more occupy the post testes space and passes between ovary, testes, seminal vesicle and reaches above seminal vesicles	Passes between testes, ovary, and seminal vesicle.	Not mention	Passes between testes, ovary, and seminal vesicle.	Passes between testes, ovary, seminal vesicle reaches above the seminal vesicle.
Vitelline follicles	originate where the ceca ends and also mark the border line between tests	Commence from post testicular to lateral fields in hind body, also appear in mid-posterior region of the body	Not mention	Vitellaria arranged laterally in posterior region of the body below the testes.	Commence from the level of testes, arranged in posterior region of the body.
Eggs	0.07-0.08 by 0.040-0.042, small, double walled.	Not mention	Not mention	0.06-0.07 by 0.02-0.03	0.06-0.07 by 0.037-0.04

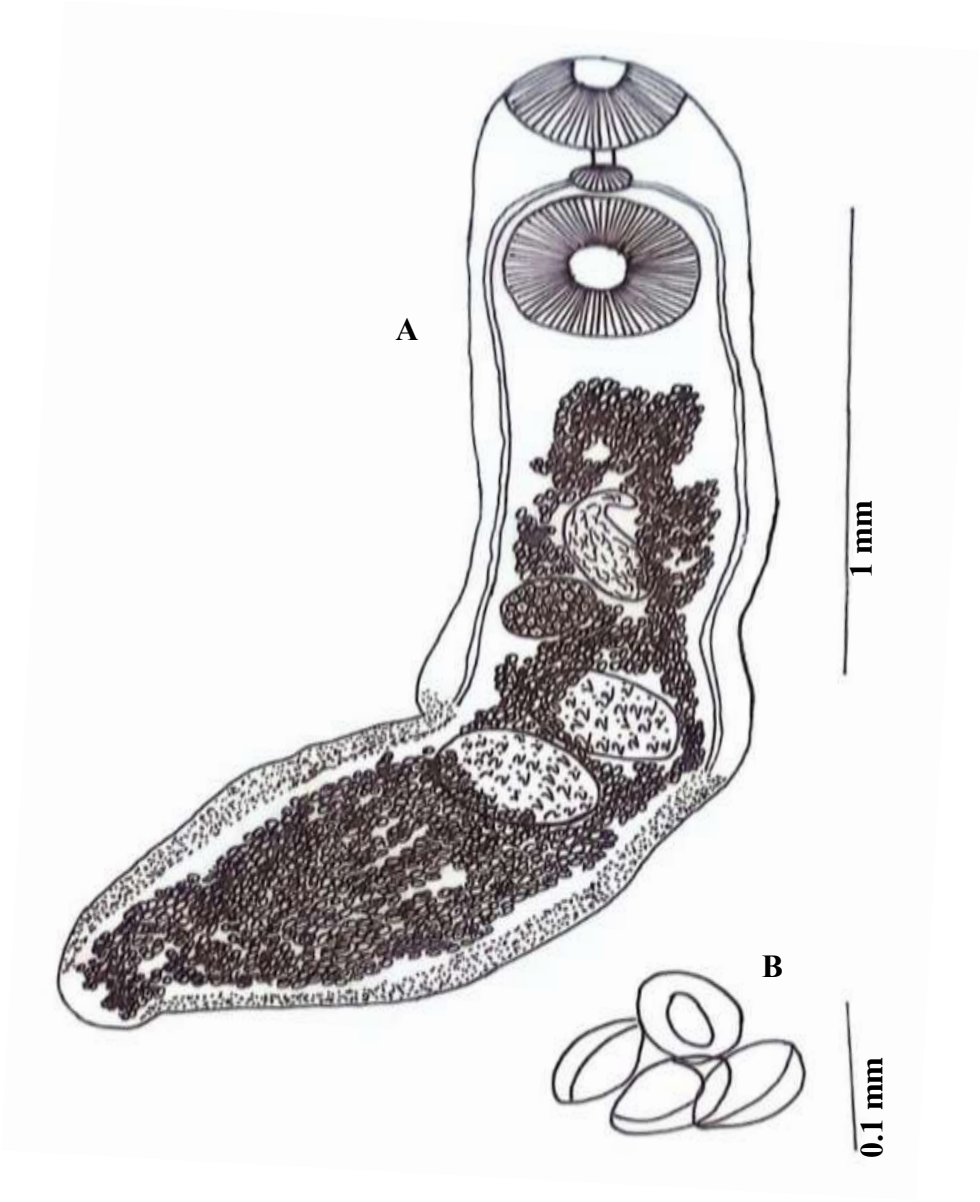

Fig. 1. ***Knipowitschiatrema sternulai*** **(A-C)**

A. ***Knipowitschiatrema sternulai*** **entire worm**
B. Eggs enlarged

Ovary small, oval, towards the left side of seminal vesicle, pre-testicular and measures 0.15-0.19 (0.149) by 0.13-0.15 (0.119) in diameter.

Testes are tandem, unequal, post-ovarian and present in the posterior region of body. Anterior testis is roughly oval to spherical in shape and 0.095-0.14 (0.104) by 0.19-0.25 (0.195) in size, while posterior testis is oval shaped, larger than anterior testis and 0.18-0.24 (0.20) by 0.21-0.25 (0.21) in diameter.

Vitelline follicles originate where the caeca ends and also mark the border line between tests in the posterior region of the body, arranged laterally and terminate almost to the posterior end of body.

Uterus more occupies the post testes space and turns between the testes, ovary and seminal vesicle and opens into the genital opening located above the seminal vesicle and below the acetabulum.

Eggs numerous, small, double walled and measures 0.07-0.08 (0.076) by 0.040-0.042 (0.038) in size.

C. *Knipowitschiatrema sternulai*
Entireworm Photomicrograph 90x

DISCUSSION

Type species *Knipowitschiatrema nicolai* Issaitschkow, 1927 was recovered in *Larus argentatus* from Russia. Larva of type species was also recovered from the gills of *Belone acus* Black sea.cf. Butskaia, 1952.

Other species include; *K. echinatum* Timon-David, 1955 in *Larus argentatus michaellis* and *L. fuscus* from France and England; *K. pakistanensis* Bushra *et al*., 2015 and *K. sternulai* Bushra *et al*., 2016 in *Sternula albifrons* from Hyderabad, Sindh, Pakistan.

Body size in present specimens are (1.71-2.31 by 0.53-0.67) nearly similar to *K. sternulai* (1.66-2.27 by 0.51-0.64) and smaller than *K. nicolai* (3.126-3.54 by 0.473-0.524); *K. echinatum* (4.6-7 by 0.49-0.69), while body size of *K. pakistanensis* (2.89-3.25 by 0.36-0.4) is larger in length and smaller in width than the present specimens.

In present specimens oral sucker is terminal, smaller as compared to ventral sucker, wider than long and measures 0.13-0.17 by 0.23-0.29 differs from *K. nicolai* in having sub-terminal oral sucker , larger than acetabulum (size not mention); *K. echinatum* (size and position not mention); *K. pakistanensis* the oral sucker is terminal, elongated, smaller than ventral sucker and measures 0.13-0.16 by 0.17-0.19, while in *K. sternulai* oral sucker is terminal, smaller than acetabulum, broader than long as in present specimens and measures 0.11-0.15 by 0.22- 0.27.

Pre-pharynx in present specimens is very short, whereas in *K. nicolai* it is wider than long; *K. pakistanensis* it is very long, and in *K. sternulai* it is very short and is nearly identical in size and shape to present specimens.

Present specimens are characterized by a rounded to spherical acetabulum, larger than the oral sucker, and located in the first quarter of body. In comparison, the acetabulum of *K. nicolai* is smaller, enclosed within the genital atrium and located in the second quarter of the body; *K. echinatum* (not mentioned); *K. pakistanensis* has rounded shape, larger than oral sucker, does not have a genital atrium and is located in the second quarter of the body; *K. sternulai* has a rounded shape, larger than the oral sucker and is located in the first quarter of the body.

The distance between the oral sucker and ventral sucker in present specimens is 0.048-0.09, while in *K. nicolai* (not mentioned); *K. echinatum* (not mentioned); *K. pakistanensis* 0.71-0.75 and *K. sternulai* it is 0.046-0.08.

Seminal vesicle in present specimens is elongated, small, saccular with narrow anterior portion, and opens through the genital pore below the acetabulum approximately in first quarter of the body , while in *K. nicolai* quite larger, twisted and situated immediately below the acetabulum; *K. echinatum* (not mentioned); *K. pakistanensis* is larger, flask shaped, voluminous size, situated immediately below the acetabulum and touching; *K. sternulai* it is small, elongated, saccular with narrow anterior section, and Situated far below the acetabulum.

In present specimens the distance between the acetabulum and seminal vesicle is 0.4, while in *K. sternulai* it is 0.3; in *K. nicolai*; *K. echinatum* and *K. pakistanensis* it is not mentioned.

Ovary in present specimens is oval, smaller, more towards the left side of body and present near the center of the body and similar to *K. Nicolai* in shape and position but differ in size, while in *K. echinatum* (not mentioned); in *K. pakistanensis* it is small, present in posterior part of the body and has same rounded shape; in *K. sternulai* ovary is roughly rounded in shape and nearly same size and position as in present specimens.

In present specimens the anterior testis is roughly oval to spherical in shape and smaller than posterior testis, whereas in *K. Nicolai* they are round and of equal size; in *K. echinatum* (not mentioned); in *K. pakistanensis* the anterior testis is smaller than posterior testis and roughly spherical in shape and posterior testis is oval shaped and in *K. sternulai* both testes are roughly rounded and anterior testis is slightly smaller than posterior testis.

In present specimens uterus more occupy the post testes space and also turn between gonads, seminal vesicle and opens into genital opening above the seminal vesicle and below the acetabulum as in *K. sternulai*, while in *K. echinatum* (not mentioned) and in *K. nicolai* and *K. pakistanensis* it passes between the gonads and seminal vesicle.

Vitelline follicles originate from caecal ending and testes level, in the posterior region of the body, while in *K. nicolai* starts in post testicular region to the lateral fields in hind body; in *K. echinatum* (not mentioned); *K. pakistanensis* originate below the testes, arranged in lateral field in the posterior region of the body and in *K. sternulai*, vitelline follicles starts from the level of testes, arranged laterally and closely reaches to posterior end of the body.

In present specimens the eggs are numerous, small, double walled and similar to *K. pakistanensis* and *K. sternulai* in shape but slightly larger in size while in *K. nicolai* and *K. echinatum* the shape and size is not mentioned.

CONCLUSION

The present specimens are in close resemblance with *K. sternulai* in all specific characteristics, while minor differences have noted in size of body, shape of ovary, new host and locality. The present specimens are therefore regarded as same *Knipowitschiatrema sternulai* but from a new host *Sternula saundersi* and locality Larkana, Sindh, Pakistan.

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