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MORPHOLOGICAL STUDY OF NOTOCOTYLUS JAMSHORENSIS N.SP. (TREMATODA: NOTOCOTYLIDAE) FROM MALLARD ANAS PLATYRHYNCHOS OF HAMAL LAKE OF PAKISTAN

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ABSTRACT

A total of 16 trematodes of genus Notocotylus were collected from the intestine of 12 host Anas platyrhynchos collected from Hamal Lake, Sindh, Pakistan. These trematodes were identified as Notocotylus jamshorensis n. sp. based on basic diagnostic characteristics including body shape and size, lateral rows and median rows, the arrangement of glands, shape, and size of testes and ovary, the distance between the ovary and anterior extremity of the hind body, distribution of Vitellaria and post-testicular space and other differentiating characters with its congeners. The new species name states Jamshoro city. Species of genus Notocotylus previously reported from Pakistan include N. panjnadensis Bhutta and Khan, 1975 collected from intestine of Anas crecca; N. sindhensis Khan et al., 2017 collected from intestine of Anas platyrynchos.

Keywords: *Notocotylus jamshorensis n. sp., avian trematode, Anas platyrhynchos, Hamal Lake, Sindh, Pakistan.*

INTRODUCTION

Sindh province, with splendid wetlands and lakes, has been always considered a welcoming ground for lots of birds who migrate to Pakistan from Russia and Siberia during the winters (Birmani, Dharejo, & Khan, 2011) Mallard *A. platyrhynchos* is the migratory bird that comes from Siberia to Pakistan in winter from October-March. Mallard is a member of the order Anseriformes (ducks, geese, and swans), generally bound to open waters and wetland habitats (Buriro, Birman & Shaikh, 2020). Mallard is migratory and depends upon a variety of food items during migration from Siberia to Asian states (Birmani et al., 2016). During its stopover habitats, it shares a variety of food items including small invertebrates, tadpoles, small fishes, and all types of plant materials (Buriro et al., 2016).

The Notocotylidae Lühe 1909 is an outsized, globally distributed group of digenean parasites in birds and mammals (Barton and Blair 2005). Its type genus *Notocotylus* Diesing, 1839 has a cosmopolitan distribution and includes from 48 to 63 valid species according to different authors (Kinsella and Tkach 2005; Boyce *et al*. 2012; Izrailskaia *et al*. 2019). Members of the genus are characterized by a high level of overall morphological uniformity with only a few differentiating characters traditionally used to distinguish between species. In addition, at least some species of *Notocotylus* are characterized by substantial, but insufficiently studied intraspecific variability of morphometric characteristics. *Notocotylus* spp. Demonstrate a greater specificity to the snail intermediate hosts than to avian definitive hosts (Stunkard 1959; Gonchar *et al*. 2019).

Material and methods

A total of twelve live hosts *Anas platyrhynchos* (Anseriformes, Anatidae) were collected from Hamal Lake during (2023-2024) in the winter seasons and studied for the occurrence of the helminth parasite. The in-depth investigation of internal organs revealed the presence of flukes from the intestine of the mallard. Sixteen specimens of genus *Notocotylus* were collected from the intestine of six hosts. Live *Notocotylus* specimens were put in 0.9% saline solution. Specimens were fixed in alcohol-formalin-acetic acid solution by applying some pressure on slides under the cover glass. After fixation then they were stained with borax carmine, dehydrated with a categorized alcohol solution, cleaned in oil of clove and xylene, and mounted in Canada balsam. Line drawings were prepared with the aid of Camera Lucida and Olympus BH2-DA Drawing tube attached to the microscope. Sizes are presented in micrometer (μm). Identification was done with the help of related literature. The parasites were processed as per the standard method of (Garacia and Ash, 1979; Schmidt, 1988).

TAXONOMIC SUMMARY

Name of host: *Anas platyrhynchos* (Aves, Anatidae)
Name of locality: Hamal Lake (27° 26′ 56.4″ N, 67° 37′ 55.2″ E) Sindh, Pakistan
Infection site: Intestine
Etymology: Species name represents Jamshoro city

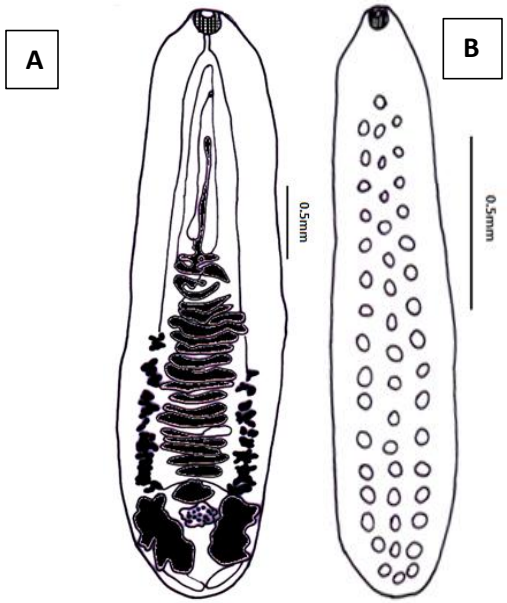
Results

Description (based on twelve trematodes)

The body is elongated flattened dorsoventrally, convex dorsally, and concave ventrally, and the anterior region is narrower as compared to the posterior end maximum width at uterine level measuring 1.49 – 3.7391 X 0.42 – 1. The tegument is unarmed. There are three rows of ventral glands, lateral rows consisting of 16 glands, and median rows consisting of 17 glands. The first median gland is positioned above the first lateral glands. Oral sucker subterminal and oval measuring 0.088 – 0.152 X 0.090 – 0.173 in size. The esophagus short measuring 0.013 – 0.108 long, bifurcates into caeca which reach up to the posterior extremity of the body below the testes. Pharynx and ventral sucker absent

The testes are lateral, lobed, indented, and longitudinally elongated situated in the posterior region of the body, extracaecal and symmetrically placed, the right testis measuring 0.25 – 0.456 X 0.10 – 0.282 while the left testis measuring 0.24 – 0.565 X 0.070 – 0.260 in size.

The Cirrus sac is very long, tubular, measuring 0.10 –1.021 X 0.05 – 0.086 in size. The genital pore is postbifurcal in position. Ovary inter-testicular is lobed in shape measuring 0.26 – 0.152 X 0.130 – 0.2282 in size, situated at the distance of 0.15–0.456 from the posterior end. Seminal receptacle is pre-ovarian, oval measuring 0.01 – 0.152 X 0.02 – 0.282 in size. Vitellaria follicular is composed of small follicles, arranged in two unequal bands, the right band is smaller than the left band situated in the posterior half of the body, reaching up to the anterior margins of the testes. The uterus is extensively thrown into 16 tightly packed transverse uterine coils. Eggs are small, filamentous measuring 0.1 – 0.4 X 0.010 –0.015 in size. Post testicular space measuring 0.054–0.173in length.



Notocotylus jamshorensis n. sp. A. General morphology and internal anatomy. B. Body outline showing the distribution of papillae. Scale bars: 0.5mm

C & D: Photograph of a total mount of anterior and posterior part of worm.

Discussion

The genus *Notocotylus* was erected by Diesing (1839) with *N. attenuatus* (Rudolphi, 1809) Kossack, 1911 from wild and domestic ducks as its type species. The genus *Notocotylus* is worldwide in distribution and contains species from various birds and mammals. Type species *N. attenuates* (Rud., 1809) Kossack, 1911. Syn. *N. triserialis* Diesing, 1839; *N. marilae* (Rud., 1819) Ditz., 1850; *N. urbanensis* of Luttermoser, 1935; *N. urbanensis* Harrah, 1922, partim- Harwood (1939); *N. thienemanni* L. et U. Szidat, 1933- Kuplianova-Shakhmatova (1959) collected from *Scolopax gallinago*; Greifswald. Also in *Mergus*, *Anas*, *Anser*, *Cygnus Mareca*, *Nettion*, *Dafila, Clangula*, *Tadorna*, *Melanitta*, *Numenius*, *Spatula*, *Somateria*, *Querquedula*, *Nyroca*, *Oxyura*, *Bucephala*, *Phalaropus*, *Phylomachus*, *Vanellus*, *Oidemia*, *Branta*, *Chen*, *Chenopsis*, *Nettopus*, *Olor*, *Gallus*.

N. attenuatus (Rudolphi, 1809) Kossack, 1911 Kee-Seon Eom, Han-Jong Rim, and Du-Hwan Jang, 1981 collected from intestine of *Anas platyrhynchos* of Korea differs from the present specimen in having smaller body size, having 14 median rows and 16 lateral rows of ventral glands, all glands are same in size oral sucker terminal, cirrus sac is club-shaped, ovary is irregularly lobed, viterllaria have equal bands uterus have 20 uterine loops, eggs are smaller in size.

N. atlanticus Horace and W. Stunkard, 1966 collected from Eider Duck *Somateria mollissima* differ from the present specimen in having a smaller body size, having 16 ventral glands in each row and median row below lateral glands, the oral sucker is circular, caeca having irregular lumina, cirrus sac is protrusible having 12 - 16 uterine loops, vitellaria having equal bands and eggs are smaller in size.

N. panjnadensis Bhutta and Khan, 1975 collected from the intestine of *Anas crecca* of Pakistan differ from the present specimen in having a smaller body size, having 12 ventral glands in each row, lateral glands are smaller than median rows, oral sucker spherical, vitellaria having equal bands uterus thrown into 20 tightly packed coils, eggs are smaller in size.

N. solitaria Kunwar Suresh Singh, 1954 collected from *Anas acuta* (pintail duck) of India differs from the present specimen in having a smaller body size, lateral rows containing 15 glands, and a middle row containing 14 rows of ventral glands, too many lobed testes, deeply lobed ovary, vitellaria having equal bands, uterine coils are not mentioned, eggs are smaller in size.

N. sindhensis Khan *et al*., 2017 collected from the intestine of *Anas platyrynchos* of Pakistan differs from the present specimen in having smaller body size, presence of pharynx, median row contain 8 rows and lateral rows contains 11 rows of ventral glands, testes slightly lobed, cirrus sac is globular roughly rounded, ovary deeply lobed, Mehlis gland irregular, uterine loops are not mentioned, eggs are smaller in size.

N. primulus Julia *et al*., 2019 collected from crested duck *Lophonetta specularioides* differs from the present specimen in having smaller body size, maximum width at the level of ovary, tegument covered with brush-tip-like spines, near oral sucker spines are tiny and also pointed but dorsolateral spines are wider and ventral spines are thinner, lateral and median spines are 13-15 rows of ventral glands, oral sucker circular with around 20 spines, genital pre-bifurcal, the ovary is bilobed, the uterus contains 30-37 coils, eggs are smaller in size.

N. chionis Capasso *et al*., 2020 collected from shorebirds differs from the present specimen in having margins scalloped from the level of the beginning of the uterus to the end of the body with larger body size, on average 25 papillae in each row, middle row with up to 27 papillae, lateral rows with up to 28 papillae, Oral sucker rounded and smaller in size, Ceca long, curving between ovary and testes, nearly reaching the posterior end of body, Cirrus sac straight, Cirrus sac broad posteriorly and sharply narrowed anteriorly, containing saccular internal seminal vesicle, Uterus long, sinuous, extending anteriorly from ovary and consisting of 24 to 31 transverse coils, eggs are smaller in size. The comparison of present species with its congener species is given in (Table 1).

Table 1. Comparative morphological measurement and features of *Notocotylus* species

Species	Present species	*N. chionis* Capasso et al., 2020	*N. panjnadensis* Bhutta and Khan, 1975	*N. sindhensis* Khan et al., 2017	*N. solitaria* Kunwar Suresh Singh, 1954
Body size	Convex dorsally & concave Ventrally 1.49 - 3.7391X0.42-1	Body margins scalloped 2.300–4.100 X 0.400–1.100	Dorsoventrally flattened 2.181X0.515	1.49 –1.64 by 0.42 –0.46	2 X0.45
Oral Sucker	Oval 0.088-0.152 X 0.090-0.173	Rounded 0.250–0.400 X (0.150–0.400	Subtermimal 0.117 X 0.127	Terminal 0.088 –0.092 by 0.090 – 0.094	Subterminal 0.08
Pharynx	Absent	-	-	Present 0.030 –0.049	-
Esophagus	0.013-0.108	-	0.078	-	short
Lateral rows	16	25	12	11	15
Median rows	17	27	12	8	14
Right testis	Lobed 0.25-0.456X0.10-0.282	0.150–0.450 X 0.190–0.450	0.235 X 0.156	Slightly intended 0.25-0.29 X 0.10-0.13	0.24- 0.25 X0.11-0.2
Left testis	0.24-0.565X0.070-0.260	0.190–0.450 X 0.110–0.230	0.245 X0.147	Slightly intended 0.24-0.28 X 0.070-0.14	0.24- 0.25 X0.11-0.2
Cirrus sac	0.10-1.021X0.05-0.086	broad posteriorly and sharply anteriorly 0.830–1.350X 0.075–0.170	0.646	globular roughly rounded 0.10-0.12 X 0.05-0.06	Long & slender 0.704 X 0.13
Genital pore ovary	Post bifurcal Intertesticular, lobed 0.26-0.152 X0.130-0.2282	- elongated longitudinally 0.110–0.230 X 0.090–0.200	- 0.156 X0.117	- deeply lobed 0.26-0.32 X 0.13-0.15	- Deeply lobed 0.144 X0.144
Seminal receptacle	Oval 0.01-0.152 X0.02-0.282	Elongated -	0.098 X0.117	Irregular in shape 0.12-0.13	0.14
Uterine coils	16	24-31	20	-	12
Eggs	0.1-0.4 X 0.010-0.015	15–20 X 11	0.010 – 0.015	0.010-0.014 X 0.007-0.009	0.0316 X0.0069

Host	Mallard	Shore bird	Eurasian teal	Mallard	Puntail duck
Location	Intestine	Intestine	Intestine	Intestine	Intestine
Locality	Hamal Lake	southern Patagonian wetlands	Panjnad Headworks area	Tando Muhammad Khan	Lucknow

Conclusion

Genus *Notocotylus* recorded for the second time from avian host Mallard, *A. platyrhynchos*. The new report will help to understand the diversity of trematodes in the avian fauna of Pakistan

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