

# Helminthological Studies of Black-winged Stilt *Himantopus himantopus* L. (Charadriiformes, Recurvirostridae) in Sindh, Pakistan

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(received June 1, 2020; revised November 2, 2020; accepted December 7, 2020)

**Abstract.** The study dealt with the helminthic fauna infecting Black-winged stilt, *Himantopus himantopus* (L.) (Charadriiformes, Recurvirostridae) from Sindh, Pakistan, includes the investigation of new varieties of helminths in wader birds of Sindh and the assessment of prevalence and the rate of infection in Black-winged Stilt, during August-November 2018. A total of 12 birds were captured from various areas of Sindh province, in which 9 birds were found infected with helminthes with total prevalence and the rate of infection were found 75% and 0.77, respectively. At present, cestodes *Himantocestus giganticus*, *Infula burhini*, *Diplophallus andinus* and a female nematode of *Amidostomum* species were recovered, with species wise prevalence and rate of parasitic infection were calculated for *D. andinus* 80% and 2, respectively for *H. giganticus* 77.77% and 2.285 for *Infula burhini* 100% and 2 and for *Amidostomum* species 80% and 3, respectively. *Diplophallus andinus* was first time reported in the present host, while more investigation for nematodes is required.

**Keywords:** cestodes, *Himantopus himantopus*, nematodes, Pakistan

## Introduction

The Black-winged Stilt *Himantopus himantopus* (L.) (Charadriiformes, Recurvirostridae) is widely distributed wader bird in tropical and sub-tropical regions of the world (Diallo *et al.*, 2019; Iqbal *et al.*, 2011; Stroud *et al.*, 2004; Boyd, 1987; Pierce, 1985). It is also a common long-legged wading bird of Sindh, Pakistan, eats a wide range of food included aquatic bugs, small tadpoles, small fish, fly larvae, crustaceans and snails which cause them a highly vulnerable to many helminthic infections (Haukisalmi, 2015; Enz and Canaris, 2008; Baker and Thomas, 2003; Pierce, 1996; Ali and Ripley, 1983; Ahern and Schmidt, 1976). In Sindh, these birds are commonly found in fish ponds, marshy areas, banks of canals and irrigated fields and are playing the role of intermediate host for many worms (McDonald, 1998).

The black-winged Stilt has been recognized for its helminths infection throughout the world that explains the high capability of this bird to carry various helminthic parasites in it without the emergence of clinical signs (Banerjee and Subramanian, 2015). Though the bird is

a definite host of many species of helminths including cestodes, trematodes, nematodes and other metazoans ectoparasites, it has not been studied for its complete parasitic fauna in the region particularly in Sindh, except a few studies (Hattar *et al.*, 2020; Siyal *et al.*, 2016; Dharejo, 2006). Therefore, a parasitic faunal study was carried out in the present study, the quantitative analysis of recorded helminths species of Black-winged Stilt was also calculated to check out the rate of infection, prevalence and intensity in this bird, hence this is a contribution towards the main theme of the study in the region.

## Material and Methods

A total of 12 birds were captured from various localities of lower Sindh, Pakistan from August to November 2018. Birds were anesthetized to dead with ethylacetate (10%) and left to be settled; dissected and examined thoroughly under the Optika Stereo microscope (Lab-20) to find out the live cestodes and nematodes. After dissection, all organs were separated from the body and kept in saline water (0.9%). The contents of the alimentary canal were cleaned and examined very carefully because most of the cestodes are found in the

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intestine. The cestodes were dehydrated in graded series of ethanol from 10 to 70%, followed by staining in alcoholic borax carmine for 30 min. The excess stain was removed by washing twice with 70% ethanol. The staining makes organs of cestodes clear, further dehydration was continued with 80% to absolute ethanol and cleaned with clove oil very carefully as the cestodes are very delicate bodies. Finally, specimens were fixed with xylol and mounted in Canada balsam permanently on microscopic slides. The chemical reagents were used from the international companies including ethanol absolute from Merck-Sigma Aldrich, USA and glycerin, clove oil, xylol and Canada balsam were used from Dae-Jung Chemicals, Korea.

The nematodes were mostly found in gizzard proventriculus, hence they were examined very carefully and removed live from the organs in saline water. Initially, all nematodes were put in warm ethanol (50%) to make them straight and dead which preserved in ethanol (70%) and glycerin (2 drops/5 mL etOH) solution. For mounting of nematodes, the temporary mounts were prepared in pure glycerin on the slide.

The identification of cestodes and nematodes were confirmed by the literature available (Anderson, 2000; Leyva *et al.*, 1980; Yamaguti, 1961; 1959). The illustrations were made with the help of Olympus CH-20 light microscope with the Olympus UD-A drawing tube attachment and measurements were taken by stage micrometer in millimeter (mm).

The prevalence and rate of infection was calculated to understand the parasitic burden in these birds (Musa *et al.*, 2012; Muhairwa *et al.*, 2007). The following formulae were used for prevalence and rate of infection: For overall prevalence (%) of the infection in birds:

$$P = N^h/N^H \times 100$$

For parasite species wise prevalence (%) in birds:

$$P = N^{hp}/N^{HP} \times 100$$

For rate of infection for each species:

$$R^i = N^p / N^{HP}$$

where:

P = prevalence in percentage;  $N^h$  = number of host birds with parasitic infection;  $N^H$  = number of host birds examined in total;  $N^{hp}$ : number of host birds with a particular parasite species;  $N^{HP}$  = number of host birds

with all parasitic infection;  $N^p$  = number of parasites of a particular helminth species;  $R^i$  = rate of Infection.

## Results and Discussion

During the present study, three species of cestodes, *Himantocetus giganticus* Mohanimad (2000) (family Diploposthidae), *Infula burhini* by Burt (1939) (family Dioecocestidae) and *Diplophallus andinus* by Voge and Read (1953) (family Acolidae), whereas one nematode of the genus *Amidostomum* species (family Amidostomatidae) were recovered from 9 host birds, *H. himantopus* (L.) from Sindh province, Pakistan. The overall prevalence of the parasitic burden in the present bird was calculated by 75% and the rate of infection was found 0.77. The parasitic species wise burden and rate of infection in parentheses for each species were also calculated as 80% by *Diplophallus andinus* (2.0) and *Amidostomum* species (3.0), 77.77% by *Himantocetus giganticus* (2.285), whereas 100% by *Infula burhini* (2.0), showing the moderate to heavy infection in the Black-winged Stilts in Sindh, Pakistan.

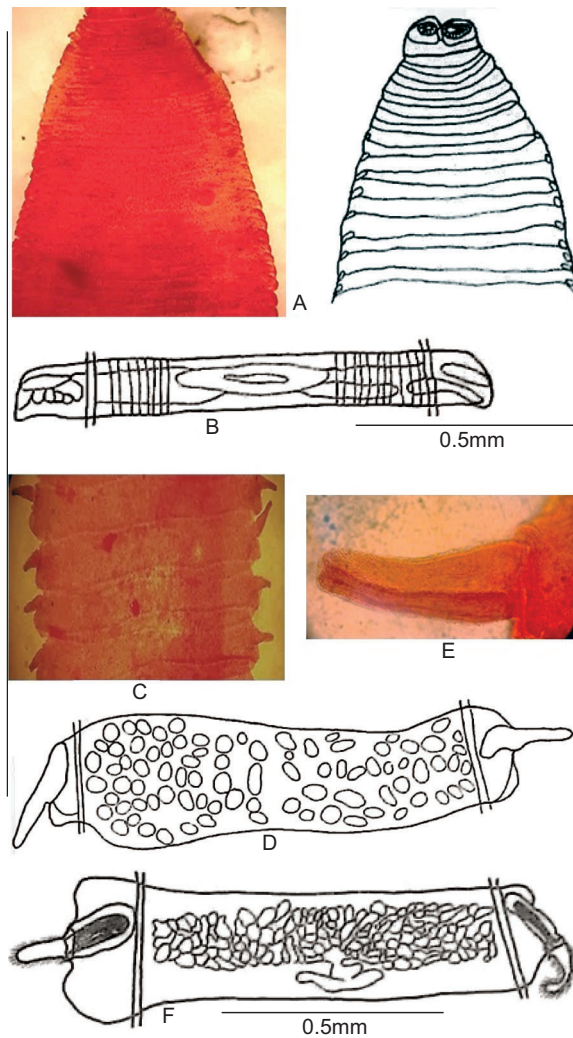
The two cestodes, *Himantocetus giganticus* and *Infula burhini* were previously reported from Black-winged Stilt (Mohanimad, 2000; Burt, 1939) however, *Diplophallus andinus* was reported for the first time from this bird, hence made a new host and new locality record.

### **Himantocetus giganticus. Mohanimad (2000) (Fig. 1A-F and Table 1), (Cestoda: Diploposthidae).**

**Materials examined.** Sixteen specimens from 7 birds; ex. *H. himantopus* (L.); small intestine; district Dadu, Sindh, Pakistan samples collected.

**Description.** The scolex small and bilobed, followed by a small neck, measured 0.18 (0.16-0.21) long, 0.46 (0.45-0.47) wide, rostellum ctenoid, measured 0.095 (0.07-0.12) long, 0.095 (0.07-0.12), the suckers large, semicircular, measured 0.08 (0.07-0.09) long, 0.17 (0.14-0.20) wide, immature segments are primitive for male organs, testis rounded and approximately 46 in numbers, arranged as bundles in median part of each segments, measured 0.19 (0.16-0.23) long, 0.27 (0.14-0.40) wide, strobilus is thick and broad, measured 0.12 (0.11-0.14) long, 0.665 (0.12-1.21) wide, all segments longer and wider; genital pore bilateral, situated at slightly mid posterior of the margin of each mature segments, two cirrus pouches situated on either sides of each segment, in immature segments cirrus pouch

extended beyond the excretory canals and in mature segments it touches the canals, in gravid it does not touch the canal, cirrus large, conspicuous, protruded and averted in immature segments, measuring 0.72 (0.56-0.88) long, 0.15 (0.14-0.16) wide, bearing fine set of spines, in mature segments these measure 0.38 (0.24-0.52) long, 0.15 (0.14-0.16) wide, vitellary glands



**Fig. 1.** *Himantocestus giganticus* Mohanimad, 2000: (A) Scolex microphotograph of anterior portion with few immature segments 4×10 (left) and Illustration (right), same scale as B, (B) Immature segment illustrated, (C) Microphotograph of few mature segments 4×10, (D) A mature segment illustrated, same scale as F, (E) Microphotograph of a cirrus pouch enlarged 10×10, (F) Gravid segment in detailed illustration.

compact, spherical, anterior to ovary, measure 0.09 (0.04-0.14) long, 0.43 (0.33-0.53) wide, ovary bilobed, medially present near the posterior margin of segment and measures 0.25 (0.23-0.27) long, 0.69 (0.67-0.73) broad, uterus developed, simple transverse, narrow tubes, stretched across the middle of the segment, bearing large sac like diverticulitis at anterior and posterior margin, mature eggs oblong to oval, measured 0.205 (0.19-0.22) long, 1.16 (1.06-1.26) broad.

**Remarks.** The genus *Himantocestus* was first introduced by Ukoli in 1965, for new species of the tapeworm, *H. blanksoni* based on the main taxonomic features in the family Diploposthidae. Spasski and Spasskaya in (1968) were disagreed with the suggestions of Ukoli due to insufficient morphological description and redescribed it (Sills, 1981), considering Diploposthidae as a synonym of Hymenolepididae. In (2000), Mohanimad introduced another new species of this genus, *H. giganticus* from *Recurvirostra avosetta* and also from *H. himantopus*, which were reported as a new host for this species.

The present tapeworm has been identified by its rostellum knob-like and neck almost absent, hooks were not seen due to poor staining in the present specimens, double cirrus sac present in each segment on lateral sides, vitelline glands ovate, narrow at anterior of the bilobed ovary, vagina absent, cirrus conspicuous and projected outwards, testis sub-oval, in clusters, each containing about 46 testes in the middle, strobila thick. The present species of *Himantocestus* has been compared with its closest allies in Table 1.

In Pakistan, the present species has been reported for the second time, supported by Mohanimad (2000) from the same host bird. However, the host bird has previously been investigation in Pakistan (Hattar *et al.*, 2020; Siyal *et al.*, 2016) but no cestode was reported before this study.

**Diplohallus andinus.** Voge and Read (1953) (Fig. 2A-D, Table 2 and 3). (Cestoda: Cyclophyllidea: Acolidae).

**Material examined.** Eight specimens from 4 birds, ex. *H. himantopus*, duodenum, District Naushaharo Froze, Sindh, Pakistan, in November 10, 2018.

**Description.** Scolex with four oval suckers, rostellum evident, lacking hooks, measuring 0.515 (0.51-0.52) long, 0.73 (0.71-0.75) broad, followed by a short neck that slowly thickened and broadened; immature segments distinct small and broad, measuring 0.28 (0.19-0.38) in length and 1.98 (1.35-2.61) in width, strobila elongated

**Table 1.** Comparison of *Himantocestus giganticus* (present specimen) with the other species of the genus reported from various birds species in the world

Morphological features	<i>H. blanksoni</i> Ukoli (1965)	<i>H. giganticus</i> Mohanimad (2000)	<i>H. giganticus</i> present specimens
Strobila	70-80×5.1	165×7.1	0.12 (0.11-0.14)×0.66 (0.12-1.21)
Scolex	0.46×0.8	0.48×0.68	0.18 (0.16-0.21)×0.46 (0.45-0.47)
Rostellum	0.17×0.075	0.14×0.045	6.03 (0.07-12)×0.06 (0.04-0.08)
Suckers	0.30-0.329 (0.31)	0.20-0.23×0.2-0.23	0.08 (0.07-0.09) 0.17×(0.14-0.20)
Hooks	Absent	18	Absent
Immature segment	0.21×2.77	–	11.88(0.760-23)×2.43 (1.44-3.42)
Mature segment	0.38×2.66	–	0.50 (0.42-0.57)×2.49 (2.26-2.72)
Gravid segment	0.77×2.56	–	0.6 (0.44-0.76)×3.46 (3.42-3.51)
Ovary	0.428	0.67	0.25(0.23-0.27)×0.69 (0.66-0.72)
Vitelline gland	0.102	0.02	0.09 (0.04-0.14)×0.43 (0.33-0.53)
Testis	60 (0.34)	40-52 (44)	0.19 (0.23-0.16)×0.27 (0.14-0.40)
Cirrus	0.449	0.076	0.72 (0.56-0.88)×0.15(0.14-0.16)
Cirrus pouch	0.274×0.122	0.23-6.26×0.10-0.12	0.38 (0.24-0.52)×0.15 (0.14-0.16)
Eggs	–	0.122×0.047	0.22 (0.19-0.22)×1.16 (1.06-1.26)
Host	<i>H. himantopus</i>	<i>Recurvirostra avosetta</i>	<i>H. himantopus</i>
Country	Ghana	Iraq	Pakistan

**Table 2.** Species of the cestode genus *Diplophallus* reported from different host species from various parts of the world

Species of <i>Diplophallus</i>	Hosts	Distribution	References
<i>D. polymorphus</i> Rudolphi (1819)	<i>Himantopus himantopus</i> , <i>H. mexicanus</i> , <i>Recurvirostra avosetta</i>	Asia, Europe, and Africa	Ukoli (1965)
<i>D. andinus</i> Voge and Read (1953)	<i>R. avosetta</i> <i>H. himantopus</i>	Peru	Voge and Read (1953)
<i>D. taglei</i> Olsen (1966)	<i>Lagidum peruanum</i>	Chile	Olsen (1966)
<i>D. coili</i> Ahern and Schmidit (1976)	<i>R. avosetta</i>	USA	Ahern and Schmidit (1976)

and thick, measuring 0.25 (0.16-0.35) long and 1.01 (0.99-1.03) broad, mature segment measured 0.50 (0.47-0.54) long, 3.24 (2.59-3.90) wide, gravid segments robust, adjoining segments connected by a thin layer of membranous cuticle, gravid segments thickest and hardest which results inter segmental separation of muscles, measure 0.62 (0.57-0.67) long, 3.49 (2.57-4.42) broad, two sets of testes, situated at sub-median part of the segment, muscular cirrus and vas deferens can be seen, small spherical testis contains 59-88 (83) in each segment at lateral side of ovary, measures 0.07 (0.05-0.09) long, 0.05 (0.03-0.07) broad, cirrus pouch large, inverted, measuring 0.19 (0.11-0.28) long, 0.32 (0.31-0.33) broad, vagina absent, single ovary present, measuring 0.32 (0.21-0.22) long, 1.8 (1.72-1.88) wide, vitelline gland globulated, located dorsal to the ovary, measures 0.26 (0.24-0.28) long, 0.495 (0.47-0.52) wide, two uterine loops were observed horizontally in early

mature segment, eggs measuring 0.1mm (0.04-0.06) × 0.075 (0.07-0.08).

**Remarks.** The genus *Diplophallus* was introduced first in (1900) by Fuhrmann for the known species of *Taenia polymorpha* reported by Rudolphi (1819) and (Ransom, 1909) belonging to family Acoleidae. There are four species known in the family Acolidae worldwide (Table 2) with which present specimens of *D. andinus* were compared (Table 3).

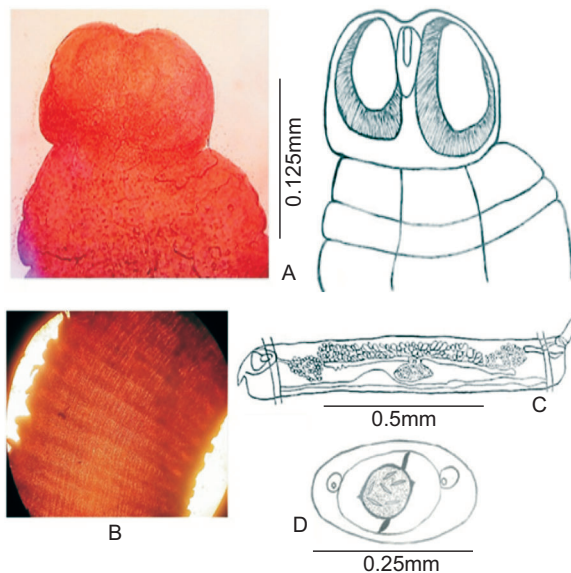
The genus is closely related to genus *Acoleus* (Voge and Read, 1953), but different in vagina that lack muscular tissues in it (Yamaguti, 1959; Wardle and McLeod, 1952). The present species of this genus well described and illustrated by Cohn (1900) and compared with *D. polymorphus* (Rudolphi) which contained armed rostellum and the longitudinal muscles in *D. polymorphus* whereas in *D. andinus*, hooks absent

and longitudinal muscles crossing the circular muscles (Wolffhugel, 1900) in addition the external transverse muscles along with layer of longitudinal muscles also present in *D. andinus*. There is evident variation in the cirrus structure of the two species. Based on these characteristics, the present specimens were identified as *D. andinus*.

Previously in Pakistan, not a single cestode was reported from re-curvirostrid bird, hence this the first time report and making new locality record for this species of cestode.

**Infula burhini Burt, 1939 (Fig. 3A-E and Table 4).** (Cestoda: Cyclophyllidae: Dioecocestidae). **Material examined.** Two specimens from 1 bird, ex. *H. himantopus*, duodenum, District Dadu and Jamshoro, Sindh, Pakistan, in November 8, 2018.

**Description.** Scolex was damage during process of mounting. The proglotids are larger and broad, mature proglotids measure 0.96 (0.87-1.05) long, 2.69 (0.3-5.09) wide, gravid segment broader, measured 2.29 (1.78-2.8) long and 1.75 (0.27-2.97) wide, genital pores situated at middle of lateral margin of proglotids, irregularly left to right and right to left, vagina in primitive form and resembles with cirrus pouch and measured 4.35 (3.5-5.25) long, 1.27 (0.86-1.68) wide,



**Fig. 2.** *Diplophallus andinus* Voge and Read (1953), (A) Scolex microphotograph 4×10 (left) and illustration (right), (B) Microphotograph of some portion of strobilus showing mature segments 4×10, (C) Gravid segment, (D) Egg enlarge.

vagina regular, fully or partially averted and similar to cirrus, armed with fine hooks, bilobed ovary composed of several follicles in adult proglotids, measuring 1.6 (1.5-1.8) long, 1.4 (0.43-2.37) wide, median vitelline gland has located dorsal to the ovary, measuring 2.1 (1.4-2.81) long, 2.16 (2.1-2.21) wide, the uterus appeared during early growth of the segment, ring shaped suppress vitelline gland and ovary, mature uterus conspicuous and having irregular diverticula, cirrus much elongate, measuring 0.735 (0.78-0.802) long, 2.93 (2.62-3.24) wide, cirrus pouch measuring 4.35 (3.5-5.25) long, and 1.27 (0.86-1.68) wide, vitelline gland in the mid-posterior to the ovary, measuring 2.10 (1.40-2.81) long, 2.16 (2.1-2.11) broad, primodia is seen before the maturation of vitelline gland and ovary.

**Remarks.** The genus *Infula* was introduced by Burt (1939) belonging to family Dioecocestidae under a type species *Infula burhini*, which was recovered from various Charadriiformes birds in Ceylon. The genus *Infula* was separated from the *Gyrocoelia* chiefly based on the armature of rostellum. It is apparent with *I. burhini* and the present specimen was recognized that not only rostellum is unarmed but the morphological variations are also seen in *Gyrocoelia* (Voge and Rausch, 1956).

The genus *Infula* is represented by two species reported worldwide, *I. burhini* by Burt (1939) and *I. macrophallus* by (Coil, 1995; Burt, 1980). The two species of *Infula* can be differentiate in number of testes, large hook-like structures in the scolex of the embryo, and suckers. In *I. macrophallus* vagina is absent, whereas *I. burhini* vagina is present. In the present specimens, vagina, a ring like uterus with huge muscular cirrus were also observed, hence this species was identified as *I. burhini* collected from *H. himantopus* and compared with its allied species (Table 4). It is based on the previous report of this species from Black-winged Stilt (Hattar *et al.*, 2020; Siyal *et al.*, 2016), this is however, first reported from the country, hence making new locality record from Sindh, Pakistan.

**Amidostomum species (Fig. 4A-G and Table 5).** (Nematoda: Strongyloida: Amidostomatidae). **Materials examined.** Twelve specimens (8 females, 4 male) from 5 birds, ex. *H. himantopus*, gizzard, district Dadu, Jamshoro and Thatta, Sindh, Pakistan, from August 11, 2018 to October 20, 2018, new host and locality record.

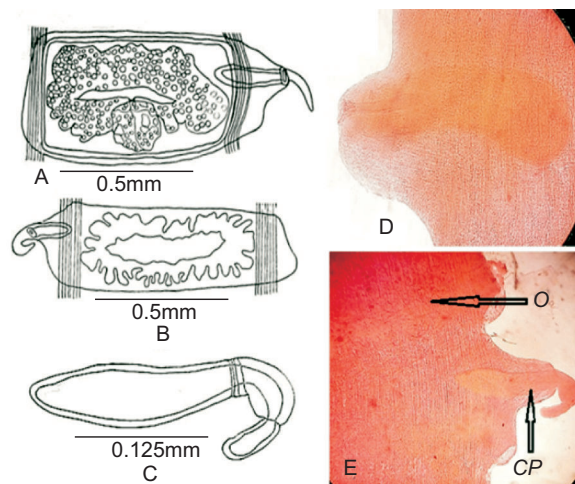
**Description. Female.** Body thin narrow, measuring 40.06 (28.12-54.01) long, buccal capsule single, large, measuring 0.35 (0.21-0.46) in length and 0.47 (0.25-

**Table 3.** Comparisons of the present specimens of *Dipllophallus andinus* with previously known species of the genus reported in the world

Morphological features	<i>D. coli</i>	<i>D. polymorphus</i>	<i>D. andinus</i>	<i>D. taglei</i>	<i>D. andinus</i>
	Ahern and Schmidt (1976)	Ukoli (1965)	Yoge and Read (1953)	Olsen (1966)	Yoge and Read (1953)
Strobila	235×4-5	120	6	9.5	0.25 (0.16-0.35)×1.01 (0.99-1.03)
Scolex	430-640×600-100	0.63×0.84	0.672×0.722	492-513×783-843	0.51 (0.51-0.52)×0.73 (0.71-0.75)
Rostellum	140×60	0.29×0.39	0.248×0.26	246-267×171-181	0.22 (0.21-0.23)×0.14 (0.12-0.16)
Suckers	250×350	0.21×0.29	0.25×0.27	375-513×783-843	0.42 (0.40-0.45)×0.32 (0.30-0.34)
Hooks	24	20-25	Absent	12	Absent
Size	115	87-122×95-121.5	-	21	0.28 (0.19-0.38)×1.98 (1.35-2.61)
Immature segment	-	-	-	-	0.50 (0.47-0.54)×3.24 (2.59-3.90)
Mature segment	-	-	0.313×0.135	-	0.50 (0.47-0.54)×3.24 (2.59-3.90)
Gravid segment	-	-	0.520×0.172	-	0.62 (0.57-0.67)×3.49 (2.57-4.42)
Neck	Absent	Absent	Absent	Present	Absent
Ovary	750-9.2	0.39-0.45	-	500	0.32(2.1-0.22)×1.8 (1.72-1.88)
Vagina	Absent	Absent	Absent	Absent	Absent
Vitelline gland	475×100-150	-	-	0.73-0.78×40-50	0.26 (0.24-0.28)×0.49 (0.47-0.52)
Cirrus pouch	350-550×125-175	0.23-0.65×0.16-0.12	-	642-802×117-139	0.09 (0.07-0.11)×0.15 (0.11-0.19)
Testis number	40-50 number	120-160	59-88×49-69	25×36.29	59-88/82 per segment
Length and width	22-40	-	-	-	0.07 (0.05-0.09)×0.05 (0.03-0.07)
Egg	73-80×49-53	39-59	33-36×43-50	73-78×40-50	0.05 (0.04-0.06)×0.07 (0.07-0.08)
Host	<i>Recurvirostra avosetta</i>	<i>R. avosetta</i> - <i>H. mexicanus</i> ,	<i>R. andina</i> ,	<i>Legidum peruanum</i>	<i>H. himantopus</i>
Site of infection	Small intestine	<i>H. himantopus</i>	<i>H. himantopus</i>		
Country	Weld county	Duodenum	Small intestine	Small intestine	Duodenum
		Asia, Europe Africa	Pampa Huaitire, Peru	Chile	Pakistan

0.65) in width, contains one large tooth, esophagus thick, muscular, measuring 0.703 (0.44-0.98) long, 0.126 (0.07-0.18) broad, eggs large, oval and thick walled, measured 0.08 (0.03-0.13) × 0.233 (0.11-0.33), vulva at posterior fifth of the body, measuring 0.056 (0.04-0.07) long, 0.683 (0.52-0.84) wide, tail is rounded, tapering to conical, measures 0.563 (0.21-0.80) long.

**Remarks.** The Genus *Amidostomum* was introduced by Linstow in (1882) and described on the basis of



**Fig. 3.** *Infula burhini* Burt, 1939: (A) Immature segment, (B) Mature segment, (C) Cirrus enlarged, (D) Microphotograph of a cirrus in mature segment, (E) Microphotograph of few gravid segments, showing mature ovary (O) and cirrus pouch (CP).

female worms only, which were recovered from Velvet scoter (*Melanitta fusca*) later by Skrjabin (1916) has redescribed it along with the male specimens recovered from the same host derived from the same host (Borgsteede *et al.*, 2006; Baylis, 1932; Cram, 1926).

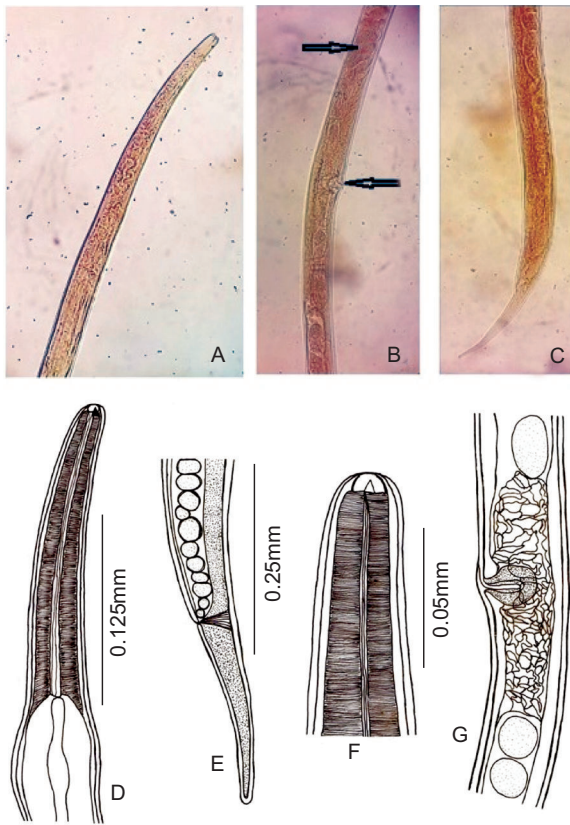
The species of the *Amidostomum* can be identified on the basis of absence of acutum, presence of single tooth, buccal capsule, esophageal bulb and presence of vulva, hence the present specimens resemble the genus *Amidostomum*. However, based on only female specimens, the species cannot be confirmed, and spicules in male specimens are specifically differentiated help in species identification, but females are almost similar, hence identified as only the genus (Table 5). It has previously been reported from *Recurvirosta avosetta*, the pied avocet and *H. himantopus* (Brogsteede *et al.*, 2006; Petrova, 1987) hence from Pakistan, it is the first report of this genus in black-winged stilt with new country record for Pakistan.

The present study shows the tapeworms and roundworms recovered from the Black-winged Stilt *H. himantopus* (Charadriiformes: Recurvirostridae) from Sindh, Pakistan. During the present study, a total of three species of cestodes were recovered from this host, including *Himantocestus giganticus* by Mohanimad (2000) *Diplophallus andinus* by Voge and Read (1953) and *Infula burhini* by Burt (1939), one species of nematodes of the genus *Amidostomum* sp. The results revealed that the present host can be infected with various types of helminths species, including cestodes, nematodes, and trematodes (Hattar *et al.*, 2020; Siyal

**Table 4.** Comparison of the present specimen of *Infula burhini* with the same and other known species reported from the world

Morphological features	<i>Infula burhini</i> , Burt (1939)	<i>Infula macrophallus</i> , Coil (1955)	<i>Infula burhini</i> Present specimen
Strobila	140-150×3.0-3.5	145×4.9	-
Scolex	0.46	0.53	-
Suckers	0.204	0.24-0.27×0.32-0.36	-
Rostellum	0.0027-0.01	0.007-0.013×0.001-0.006	-
Hooks	-	0.015×0.019	-
Neck	0.23×0.38	0.40×0.14	-
Vagina	0.47×0.15	-	5.00 (4.66-5.35)×1.9 (1.75-2.05)
Ovary	0.68	-	1.6 (1.5-1.8)×1.4 (0.43-2.37)
Vitelline gland	0.29	0.53	2.10 (1.4-2.81)×3.16 (2.21-4.11)
Mature proglotid	0.5×1.7	-	0.96 (0.87-1.05)×2.69 (0.30-5.09)
Gravid proglotid	0.8×1.9	-	0.4 (0.3-0.6)×1.75 (0.27-2.97)
Cirrus	-	-	7.35 (7.8-8.91)×5.86(2.62-3.24)
Cirrus pouch	-	-	4.35 (3.5-5.25)×1.27 (0.86-1.68)

et al., 2016; Mohanimad, 2000). The presence of three species of cestodes indicated that this host feeds on a variety of aquatic grasses, while feeding on small invertebrates like freshwater snails, crustaceans, or small fishes got attached with grasses which can be the intermediate hosts for many cestode species and carrying the cestodes larvae and hence, the birds as definitive host can also be infected with cestodes (Globokar, et al., 2017; Georgiev et al., 2005; Scholz et al., 2002; Pietrock and Scholz, 2000; Mc Donald, 1998; Pierce, 1985; Goriup, 1982).



**Fig. 4.** *Amidostomum* species Female, Microphotographs of female specimen: (A) Anterior portion of body, (B) Middle portion of body showing vulva (vl) and eggs inside uterus (E), (C) Posterior portion of body showing tail on its terminal end, (D) Detailed illustration of anterior portion, (E). Illustration of posterior portion of the body with tail, (F) Anterior portion showing buccal capsule with an acute tooth, (G) Vulva and eggs inside middle portion of the body.

**Table 5.** Comparisons of the present specimen of genus *Amidostomum* with known species of the genus in the world

Morphological features	<i>A. acutum</i> (Lundahl, 1848)	<i>A. petrovi</i> (Shakhtantskaya, 1956)	<i>A. monodon</i> (Linstow, 1882)	<i>A. fulicae</i> (Rudolphi, 1819)	<i>A. anseris</i> (Zeder, 1800)	<i>A. henryi</i> (Skrjabin, 1915)	Present specimens
Total length	7.300×11.8	10.12×16.63	16.5×140	11.5-13×0.25-0.3	21.15×0.31	14.5 (180-187)	41.06 (28.12-54.01)×0.43 (0.31-0.56)
Buccal capsule	21-26	9-13	-	0.03×0.035	-	18.5	0.33 (0.21-0.46)×0.45 (0.25-0.65)
Esophagus	90-126	620-940	55	1-1.5×0.1-0.12	-	850	0.71 (0.44-0.98)×0.12 (0.07-0.18)
Egg	70-94×38-59	73-91×40-58	92×62	0.09-0.11×0.06-0.75	0.085×0.05	92.5-103.6×70-80	0.05 (0.04-0.07)×0.68 (0.52-0.84)
Vulva	213-286	310-320	17.4	2.12×2.25	0.031×0.037	-	0.08 (0.03-0.13)×0.22 (0.11-0.33)
Tail	1.730×3.81	2.15-2.99	2.66	0.3×0.25	30×400	2.72	0.50 (0.21-0.80)×0.05 (0.02-0.09)



Among cestodes, *Himantocestus blanksoni* (Diploposthidae), *Infula burhini* (Dioecocestidae) and *Thomasitaenia nunguae* (Progynaotaeniidae) from Black-winged Stilt (Awad and Al-Muthafer, 2016; Ukoli, 1965); later reported by Mohanimad (2000) *Himantocestus giganticus* from the same host along with Black-necked Stilt (type host) from Iraq, and *Diplophallus polymorphus* was also reported from the same host in India (Kalse and Shinde, 1999; Burt, 1980).

The genus *Amidostomum* is the widest and abundantly found in the birds of family Anatidae (Bartlett, 2008; Chaturvedi and Kansal, 1977). Seurat (1918) collected *A. chevreuxi* from *Anser albifrons* and *H. himantopus* from Africa (Petrova, 1987), *A. raillieti* Skrjabin (1916) was reported from the stomach of *Fulica atra* from Egypt, Turkistan and Asia (Kavetska et al., 2011; Borgsteede et al., 2006) and different morphological forms of *A. acutum* have been reported from various birds species of family Anatidae, Tadornidae (Anseriformes), Rallidae (Gruiformes) and also from Recurvirostridae (Charadriiformes) (Borgsteede et al., 2006; Pennycott, 1998; Lomakin, 1993), in Pakistan, *A. fulicae* reported by Rudolphi in (1819) and Birmani et al. (2011) from the Eurasian black coot (*Fulica atra*) but it is not reported from the present host before this study.

In the present study, the two cestodes, *Himantocestus giganticus* and *Infula burhini* were previously reported from Black-winged Stilt, however, *Diplophallus andinus* was reported for the first time from this bird, and hence made a new host and new locality on record from Pakistan. The nematodes still need to verify further for specific identification and requires further investigation to report new records and new species infecting Black-winged Stilt.

### Acknowledgement

Authors acknowledge the Higher Education Commission (HEC), Islamabad, Pakistan for funding the Research Project 20-4514/14 under NRPU Program and the Sindh Wildlife Authority for allowing the authors to travel birds from the collection site to research laboratory and also allowed to catch the birds for research purpose.

**Conflict of Interest.** The authors declare no conflict of interest.

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