Research Article

New species and new record of the genus *Uvitellina* Witenberg, 1923 (Cyclocoelidae: Haematotrematidae) infecting Black-Winged Stilt, *Himantopus himantopus* (L.) (Charadriiformes: Recurvirostridae) in Sindh, Pakistan

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Abstract

During the helminthic investigation of wading birds of Sindh, the Black-winged stilt, *Himantopus himantopus* (L., 1758) (Charadriiformes: Recurvirostridae) was examined to update the trematode fauna of Sindh, Pakistan. The trematode worms were recovered from the body cavity of the host and identified as species of the genus *Uvitellina* Witenberg, 1923; total 22 birds were examined in which 19 birds were found infected with 56 specimens of three species under the genus *Uvitellina*. The three species were identified as *U. vanelli* (Rudolphi, 1819) and *U. pseudocotylea* Witenberg, 1923 as new host and country record, and *U. sindhiensis* was introduced as species novum, which was named on its type locality, Sindh province, Pakistan.

Keywords: *Himantopus himantopus*; new species; Pakistan; Sindh; taxonomical study; *Uvitellina*

Introduction

The Black-winged stilt *Himantopus himantopus* (Linnaeus, 1758) is a common long legged, medium sized, wading bird, belonging to avian order Charadriiformes and family Recurvirostridae. It is cosmopolitan and frequently found in Sindh near aquatic and marshy water bodies, mostly in summer to spring. It feeds on a wide range of food, including aquatic beetles, bugs, worms,

dipterans larvae, crustaceans, snails, tadpoles, small fish, etc. which make highly possible the transference of helminthic infection from primary host to the bird as secondary host [1-5].

In the present study of helminthological investigation of aquatic birds of Sindh, Pakistan, the black-winged stilt was examined for its parasitic infection in the region, especially with reference to the

trematode infection. Previously, there has no significant work been done on this bird host, except some sporadic investigations [6]. The present work is a contribution towards the addition of *Uvitellina* species that has been made in the trematodes fauna of wading birds found in Sindh, Pakistan.

Materials and methods

For helminthological examination, 22 birds of Black-winged stilts were collected and examined for their trematode infection from different localities of Sindh during November 2017 to May 2018. Birds were anesthetized with chloroform and autopsy was carried out. All the visceral organs, intestine and body cavity of each bird were thoroughly examined under stereo microscope. The trematodes under present study were recovered from the body cavity and were isolated with the help of soft brush and washed with 0.9% saline water. The trematodes were then left in saline water to inflate for 10-15 minutes; these were shifted to 10% ethanol and were processed for permanent mounts in Canada balsam through dehydration in 10-70% ethanol. At this stage the specimens were stained with borax carmine solution; further dehydration was done by 70-90% and absolute ethanol consecutively and fixed with clove oil and xylol for 1-2 minutes respectively. The mounting was carried out in Canada balsam at final stage. The illustration and measurements were made by using Olympus-UDA drawing tube attachment and oculo-micrometer respectively; measurements were taken in millimeters (mm). Specimens were deposited in the Advanced Parasitology Research Laboratory-Museum of Parasitology (APRLMP), Department of Zoology, University of Sindh, Pakistan.

Results

Uvitellina vanelli (Rudolphi, 1819)

(Digenea: Cyclocoelidae:

Haematotrephinae)

Figures 1-3, Table 1.

U. tageri Yamaguti, 1933; *U. keri* Yamaguti, 1933.

Type Host:

Vanellus vanellus Northern lapwing
(Charadriiformes: Charadriidae).

Material examined

Present Host:

Himantopus himantopus Black-winged stilt.

Locality: Sindh (District Naushehro Feroze), Pakistan.

Date of collection: 20-i-2018.

Site of infection: Body Cavity.

No of parasites recovered: 24 out of 8 birds.

Prevalence: 87.5%

Intensity: 3.42.

Status: New locality and new host record.

Description

Body is long and narrow to lanceolate, with tapered anterior end, measuring 15.07 (15.57-14.56) long and 2.87 (2.90-2.84) wide at center of the body; ventral sucker absent; oral sucker well developed, wider than long measuring 0.14 (0.14-0.15) long and 0.39 (0.49-0.29) wide; pharynx sufficiently large, 0.34 (0.30-0.39) long and 0.41 (0.40-0.42) wide; caeca wide and simple, united at posterior; uterine loops invading over caeca; vitellaria confluent posteriorly in form of small follicles; ovary round and pre-testicular, with 0.41 (0.38-0.45) long and 0.43 (0.45-0.42) wide; testes trigonal and tandem, anterior testes measuring 0.86 (0.83-0.90) long and 0.87 (0.85-0.88) wide, posterior tests 0.84 (0.90-0.79) long and 0.81(0.85-0.78) wide; eggs reniform with very thin and fragile shell, measuring 0.23 (0.30-0.23) long and 0.1(0.11-0.09) wide.

Remarks

The present species, *U. vanelli* was first introduced by Rudolphi in 1819 [7], which was recovered from its type host, *Vanellus vanellus*, under the name, *Monostoma vanelli*. This was later redescribed by Bashkirova [8] and followed the nomenclature of Rudolphi [7], however Dubois [9] described it under the genus

Uvitellina and proposed synonymies *U. kerri* Yamaguti, 1933 and *U. tageri* Yamaguti, 1933 with *U. vanelli*, because both species are identical in particular characteristics to this species. In 1971, Yamaguti [10] illustrated it as *Haematotrephus*, *Uvitellina kaniharensis* (first proposed by Gupta [11], considered this species synonymous to *Cyclocoelum* (*Haematotrephus*) *vanelli* [7, 9]. Yamaguti [10, 12] suggested that this species has genital pore is post pharyngeal, vitalline field extend posteriorly, ovary pre-testicular hence, assigned it in genus *Uvitellina*, having uterine coil invading lateral side of caeca and posteriorly most uterine coils touch the anterior to anterior testis. The present species also recovered from other host birds, which played the role of its definitive host, including *Tringa flavipes* [13], *Vanellus cinereus* [12], *Tringa melanoleuca* [14]; presently recovered from *Himantopus himantopus*. This species is verified on the basis of description, measurements, body size and shape which is large, longiform, genital pore is post-pharyngeal, uterine coil is over reaching caeca, vitallaria extend laterally to the posterior, ovary pre-testicular, testis are tandem and trigonal, above cirrus sac, hence this species has been identified as *Uvitellina vanelli*. However this species was recovered for the first time from the present host in Sindh region, therefore making new host and new locality on record.

***Uvitellina pseudocotylea* Witenberg, 1923**
(Digenea: Cyclocoelidae:

Haematotrephinae)

Figures 4-6, (Table 1).

U. pseudocotylea Witenberg, 1923.

Type Host

Himantopus candidus Long-legged Plover.

Material examined

Present Host: *Himantopus himantopus*
 Black-winged stilt (Charadriiformes:
 Recurvirostridae).

Locality: Sindh (District Dadu), Pakistan.

Date of collection: 21-iii-2018.

Site of infection: Body Cavity.

No. of parasites recovered: 18 out of 6 birds.

Prevalence: 75%.

Intensity: 6.

Description

Body longiform, anteriorly tapered and posteriorly broad, measuring 14.11 (16.54-11.68) long and 2.21 (2.52-1.90) wide at the center of the body; oral sucker well developed with 0.25 (0.23-0.28) × 0.26 (0.30-0.28); cirrus pouch measures 0.46 (0.57-0.35) × 0.20 (0.28-0.13); pharynx large and wide with 0.67 (0.35-0.32) × 0.42 (0.42-0.43); ovary rounded, measured 0.43 (0.40-0.46) × 0.46 (0.42-0.50); testes tandem and trigonal, anterior testis smaller than posterior both measured 0.67 (0.66-0.69) × 0.56 (0.59-0.54) and 0.8 (0.78-0.82) × 0.65 (0.61-0.69) respectively; egg soft with delicate shell, measuring 0.16 (0.21-0.11) × 0.05 (0.11-0.08) in size, more or less oval in shape.

Remarks

Present species of the genus *Uvitellina* was recovered from Black-winged stilt whereas it has also been previously recovered from *Charadrius dubius* (Little ringed plover) and *C. placidus* (Long billed plover (Charadriidae). Presently, it has been recovered from the body cavity of host and is verified on the basis of description, measurements, body size, which is large lanceolate and uterine loops is over reaching the ovary, slightly touching and crossing the side of ovary, vitallaria extend laterally posterior, ovary pre-testicular spherical, located at opposite to the posterior testis, testis are tandem and trigonal, hence the species was identified as *U. pseudocotylea*. However this species has been recovered from *H. himantopus* for the first time from the present host from Sindh region, hence making new host and new locality on record.

***Uvitellina sindhiensis* species novum**

(Digenea: Cyclocoelidae:

Haematotrephinae)

Figure 7-10, (Table 2).

Type Host:

Himantopus himantopus (L.) (Black-winged Stilt (Charadriiformes: Recurvirostridae).

Material examined

Present Host:

Himantopus himantopus Black winged stilt.

Locality: Sindh (District Naushehro Feroze), Pakistan.

Date of collection: 05-iv-2018.

Site of infection: Body Cavity.

No. of parasites recovered: 14 out of 6 birds.

Prevalence: 100%

Intensity: 2.33.

Type Specimens: Holotype – 01 and Paratypes – 03. Other material: 10. Deposited in APRLMP, University of Sindh, Jamshoro.

Etymology

The epithet of the present new species is given after the type locality, which is Sindh province, from where it is been recovered and introduced as new species to science, hence the name *Uvitellina sindhiensis* has been proposed.

Description

Body is large without spine anteriorly tapered and posteriorly wide and rounded in shape, measured 15.13 (15.4-14.86) long and 3.07 (3.64-2.5) wide in the middle of the body; oral sucker less developed and 0.33 (0.42-0.24) × 0.13 (0.133-0.13) in size; ventral sucker absent; pharynx larger than oral sucker and wider relatively, measuring 0.45 (0.46-0.45) × 0.40 (0.4-0.45) in size; testes rough and trigonal, anterior testes measured 1.0 (1.02-0.98) long and 0.72 (0.71-0.73)

wider; posterior testes measured 0.99 (1-0.99) long and 0.99 (1.04-0.94) wide; ovary oval, pre-testicular and measured 0.45 (0.44-0.46) in length and 0.50 (0.51- 0.50) in width; genital pore post pharyngeal in position, opens at mid line of the pharynx; vitellaria always confluent posteriorly at lateral sides of caeca; cirrus located at one side of the body; eggs spherical, measured 0.17 (0.24-0.11) long and 0.12(0.15-0.10) wide.

Remarks

In the present collection of trematodes infecting Black-winged stilt, a new species under the genus *Uvitellina* has been identified, which has been compared with its closed species including the two previously described species. Yamaguti [12] proposed eight species of the genus *Uvitellina* included *U. pseudocotylea*, recovered from *Himantopus candidus* (Long-legged plover) in Russia; however it is also recovered in the present study and find the difference in taxonomical features given in (Table 2).

The new species of the genus *Uvitellina* is different from previous species reported from various bird of Sindh, Pakistan, on the basis of morphometric characters. The genital pore is post pharyngeal opening in the center line of the pharynx and vitelline fields always united posteriorly placing in genus *Uvitellina*. Besides, the ovary of *U. sindhiensis* sp.n. is oval, pre-testicular and form triangle with testes in position, placing as new species in subfamily Haematotrematidae. On the basis of these characters the new species, *Uvitellina sindhiensis* has been proposed.

Table 1. Comparison of morphometric characteristics of different species of the genus *Uvitellina* recovered from various host species (in mm)

Name of organs	<i>U. vanelli</i>	<i>U. teesae</i>	<i>U. megacaecatum</i>	<i>U. indica</i>	<i>U. dolfusi</i>	<i>U. adelphus</i>	<i>U. pseudocotylea</i>	<i>U. macroisophaga</i>	<i>U. kaniharensis</i>	<i>U. himantopi</i>	<i>U. iraquensis</i>
Host	<i>Himantopus himantopus</i>	<i>Himantopus himantopus</i>	<i>Himantopus himantopus</i>	<i>Lobivanello indicus</i>	<i>Vanellus inereus</i>	<i>Himantopus leucocephalus</i>	<i>Himantopus acndidus</i> <i>Charadrius placidus</i>	<i>Oxyechus vociferous</i>	<i>Glottis nebularia</i>	<i>Himantopus himantopus</i>	<i>Vanellus leucurus</i>
Locality	Pakistan	Pakistan	Pakistan	India	Nanjing	Australia	Russia, Turkistan, Japan	California .USA	India	Ukraine	Iraq
Body size	15.07(15.57-14.56)×2.87 (2.90 -2.84)	7.89×1.49	(6.482) 5.36-8.25	5.683-7.656×1.113-1.536	14.000	8.100×14.820	6.5v2,4	7.8 (6-9)	13.44-16.128x×2.88 -3.84	13,644×15,100	8,834×10,459
Oral sucker	0.14 (0.14 - 0.15)×0.39 (0.49 -0.29)	360	(0.27) 0.32-0.50	210×326	--	--	None	210	272-336	408 (380-450)	363 (290-420)
Pharynx	0.034 (0.30 - 0.39) ×0.41 (0.40 -0.42)	416	Absent	249×345	483	500	262×331	155(143-171)	288×355	383 (370-395)	323(295-345)
Testes	Ant. 0.086 (0.83 -0.90)× 0.87 (0.85 – 0.88) Post.0.84 (0.90 - 0.79)×0.81 (0.85 -0.79)	433×533	Ant.0.38 (0.33-0.507)× 0.501 (0.391-0.724)	384×268	955 (920-989)	690	661×1,254	461 (414-628)	528×1,040	626 (470-710)	513(300-690)
Ovary	0.41 (0.38- 0.45) × 0.43 (0.45 - 0.42)	299	0.31-0.144 (0.4405) × 0.22 -0.376 (0.3143)	210×268	529	350	319×513	191 (142-242)	320×336	425 (410-485)	289 (240-340)
Egg	0.23 0.300.23)×0.1 (0.11 -0.09)	145 (136-153) 55 (50-59)	0.131 (0.13-0.144)× (0.054) (0.05-0.057)	145(136-153)× 55(50-59)	243	175 (170-180)× 75 (70-80)	147 (134-159) 55 (48-62)	136 (128-143)×50-(43-57)	--	183 (150-195) 98 (90-105)	205 (193-215) 72(63-88)

Table 2. Comparison of morphometric characteristics of new species of the genus *Uvitellina* with its closely related species infecting various host birds, all measurements are given in mm.

Name of organs	Present new species	<i>U. teesae</i>	<i>U. megacaecatum</i>	<i>U. indica</i>	<i>U. dolfusi</i>	<i>U. adelphus</i>	<i>U. vanelli</i>	<i>U. pseudocotylea</i>	<i>U. macroisophaga</i>	<i>U. kaniharensis</i>	<i>U. himantopi</i>	<i>U. iraquensis</i>
Host	<i>Himantopus himantopus</i>	<i>Himantopus himantopus</i>	<i>Himantopus himantopus</i>	<i>Lobivanellus indicus</i>	<i>Vanellus cinereus</i>	<i>Himantopus leucocephallus</i>	<i>Vanellus vanellus</i>	<i>Himantopus candidus</i> <i>Charadrius placidus</i>	<i>Oxyechus vociferous</i>	<i>Glottis nebularia</i>	<i>Himantopus himantopus</i>	<i>Vanellus leucurus</i>
Locality	Pakistan	Pakistan	Pakistan	India	Nanjing	Australia	Veina	Russia Turkistan Japan	California USA	India	Ukraine	Iraq
Body size	15.13(15.4-14.86) 3.07 (3.64-2.5)	7.89×1.49	5.36-8.25 (6.482)	5.683-7.656×1.113-1.536	14.000	8.100×14.820	2 0, 400	6.5×2.4	7.8 (6-9)	13.44-16.128×2.88-3.84	13,644×15,100	8,834×10,459
Oral sucker	0.33(0.42-0.24) 0.13(0.13-0.13)	360	0.32-0.50 (0.27)	210×326	None	None	None	None	210	272×336	408 (380-450)	363 (290-420)
Pharynx	0.45(0.46-0.45) 0.40(0.4-0.45)	416	Absent	249×345	483	500	500	262×331	155(143-171)	288×355	383 (370-395)	323(295-345)
Testes	Ant.1 (1.02-0.98) 0.72 (0.71-0.73) Post.0.99 (1-0.99) 0.99 (1.04-0.94)	433×533	Ant.0.33-0.507 (0.382)×0.391-0.724 (0.501)	384×268	955 (920-989)	690	690	661×1,254	461 (414-628)	528-1,040	626 (470-710)	513(300-690)
Ovary	0.45(0.44-0.46) 0.50 (0.51-0.50)	299	0.31-0.144 (0.4405)×0.22 - 0.376 (0.3143)	210-268	529	350	350	319×513	191 (142-242)	320-336	425 (410-485)	289 (240-340)
Egg	0.17 (0.24-0.11) 0.12 (0.15-0.10)	145 (136-153)×55 (50-59)	0.13-0.144 (0.131)×0.05-0.057 (0.054)	145(136-153) × 55(50-59)	243	175 (170-180) ×75 (70-80)	175 (170-180) × 75 (70-80)	147 (134-159) × 55 (48-62)	136 (128-143)×50-(43-57)	-	183 (150-195)×98 (90-105)	205 (193-215)×72(63-88)

Fig. 1

Fig. 2

Fig. 3

Figures 1-3. *Uvitellina vanelli* (Rodulphi, 1819): 1. Whole specimen, 2. Posterior portion of the body showing position of gonads and vitalleria, 3. Mature egg

Fig. 4

Fig. 5

Fig. 6

Figures 4-6. *Uvitellina pseudocotylea* Witenberg, 1923: 4. Whole specimen, 5. Posterior portion of the body showing position of gonads and vitalleria, 6. Mature eggs

Fig. 7

Fig. 8

Fig. 9

Fig. 10

Figures 7-10. *Uvitellina sindhiensis* sp.n. 7. Whole specimen, 8. Anterior portion of the body showing weak oral sucker and cirrus pouch, 9. Posterior portion of the body showing position of gonads and vitalleria, 10. Mature eggs

Discussion

The trematode family Cyclocoelidae Stossich, 1902 consists of six subfamilies are latest recognized largely based on the orientation of the ovary relative to the testes. Subfamily Cyclocoelinae [15] has a characteristic of the ovary, located between testes (inter-testicular) make a triangle with the testes; Haematotrephinae [16] has the orientation of the ovary is located at upper part of anterior testes (pre-testicular) to opposite to the anterior testis forming a triangle with the testes; in subfamily Ophthalmophaginae [17] the ovary is post-testicular forming a straight line with the testes; in Hyptiasminae [16], the ovary is inter-testicular and the testes are tandem to nearly tandem; in Skrjabinoceolinae [18], the ovary is inter-testicular and the testes are nearly side by side; in Szidatitreminae [18] the position of the ovary ranges from being post-testicular to opposite to the posterior testis forming a triangle with the testes. On the basis of these characteristics, the species in genus *Uvitellina* in the present study were described.

The family Cyclocoelidae has commonly related and dependable features which are useful to determine the subfamilies and its genera. Most of the species lack an oral sucker and few are known to have it but less developed, and these contribute to broad range of variation seen in detailed measurement as well as its location [19, 20]. Dronen and Blend [21] described major three features to describe genera and subfamilies of family Cyclocoelidae, including posterior extension of vitalline fields may be reaching posteriorly, position of ovary in relation to testis and location of the genital pore related to the pharynx.

In the region, especially in Pakistan, the studies on this family and genera have been remained a controversial in identification, hence many of the species names are synonymized and close similarities within the

species always have shown a confusion in determination of cyclocoelid species. However Dronen and Blend [21] have made an effort to remove the confusion with in the species morphology, which here remained supportive measure to identify the genus and its species.

The genus *Uvitellina* was first introduced by Wittenberg in 1923, reported from various birds of the order Charadriiformes, under the family Cyclocoelidae and subfamily Haematotrephinae. The family Cyclocoelidae was known accidental parasites of various birds; until parasites found in respiratory system, especially lungs and air sac [22-26]. Previously, *Uvitellina indica* was collected from *Vanellus indicus* was described from India [27], but under *Lobivanellus indicus*, later it was redescribed under the genus *Uvitellina* with *U. kaniharensis* [11], which was recovered from *Tringa nebularia*, *U. keri* [12] from *V. cinereus* and *U. tageri* [12] and *U. vanelli* [7] from *V. vanellus* in Japan, *U. macroisophaga* [28] from *Charadrius vociferious* in U.S. and *U. magniembria* [29] from *Himantopus candidus* in Russia. Johnston [30] elaborated *Haematotephus adelphus* from *Himantopus leucocephalus* however Witenberg [29] has transferred in to genus *Uvitellina* as *U. adelpha*, because vitalline fields of this species were reaching posteriorly and connecting to each other. Yamaguti [10] kept this species in the genus *Haematotephus* but the original description of it strongly showed the vitalline fields were reaching posteriorly and hence it was designated as the species of genus *Uvitellina* [21-25].

In the region of western to southern-east including Pakistan, *U. iraquensis* was reported from Iraq, infected to *V. leucurus* [31] and two species were reported from Pakistan including *U. teesae* from *Butastur teesae* [32] and *U. megacaecatum* from *Himantopus himantops* [6]. Dronen and Blend [21] have rescribed *U. teesae* Dharejo et al. [32] under

the genus *Haematotrephus* on the basis of incomplete description and synonymized it as *H. teesae*. Dharejo *et al.* [32] have illustrated the vitalline field as vitallaria occupied extra-caecal lateral fields, suggested not extended posteriorly, which is the key character of the later genus [32].

The present study hence is the connection of more fauna of trematodes of marshy and semi aquatic birds include three more species in Sindh region. It deals with the trematode infection and burden on host and the prevalence of trematodes seem to be higher in black-winged stilt, *H. himantopus* with *U. vanelli* found more noticeable species in the host. Presently, *U. vanelli* and *U. pseudocotylea* were reported from the present host for the first time in the country hence making new host for both the species on record and a new species with the evident morphological differences and comparison (Table 2 and 3) with known species, following the key given by Dronen and Blend [21] was introduced in the region and proposed as *U. sindhiensis* sp.n.

Authors' contributions

Conceived and designed the experiments: S Naz & NA Birmani, Performed the experiments: FA Hattar & NA Birmani, Analyzed the data: FA Hattar & S Naz, Contributed materials/ analysis/ tools: S Naz, Wrote the paper: S Naz & FA Hattar.

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