

Original Article

Heterotestophyes gibsoni sp.n. (Trematoda: Heterophyidae) from the bird little tern (*Sternula albifrons*) in Sindh, Pakistan

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Abstract

During an investigation on helminth parasites of birds, a new species of trematode genus *Heterotestophyes gibsoni* sp. n. was recorded from the intestine of little tern (*Sternula albifrons*) collected from Jamshoro, Sindh, Pakistan. The new species is characterized by having small body, maximum width attained at the level of mid body region and twisted at the level of pharynx. Oral sucker terminal, rounded and broader than long. Esophagus long, intestinal bifurcation is above the acetabulum. Ventral sucker muscular, rounded, occupy 2nd. quarter of the body. Testes lie in posterior most region of the hind body, these are Juxta-opposite, rounded in shape. Genital atrium occupies middle of the body, elongated to rounded in shape, distinctly separated from acetabulum, spines in the crown are 57-60 in number. Ovary pre-testicular, sub median, equal in size to anterior testis. Uterus profuse with loops in hind body, between the genital atrium and anterior testis. Vitellaria follicular, commence at little distance below the genital atrium, extend up to the hind body above the testes, eggs are oval shaped and double walled.

Key words: *Heterotestophyes*, heterophyidae, *Sternula albifrons*, Jamshoro, Sindh, Pakistan.

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INTRODUCTION

Birds are in general source of dissemination of parasites, causing fatal diseases in humans, both protozoans as well as helminth parasites (McLaughlin, 2001). The presence of helminths on large scale causes losses to animal husbandry and also results in the direct economic loss through mortalities and egg production decreased or suspended in birds (Soulsby, 2006). Like other animals, man also harbour both protozoan and helminth parasites.

These parasites cause severe infectious diseases in humans if not treated, may result in death of their host (Roberts and Janovy, 2009). The problem of helminthic parasitism is not only in Pakistan but also in many countries of the world is of great economic importance and deserves serious attention. The species of the genus *Heterotestophyes* (Leonov, 1957) is recovered from the small intestine of the host little tern (*Sternula albifrons*) in Jamshoro, Sindh,

Pakistan. It belongs to the family Heterophyidae (Leiper, 1909). The family Heterophyidae is a large family and contains about thirty-five genera and species mainly from avian hosts (Yamaguti, 1971; Bray *et al.*, 2008).

Literature reveals only two species of the genus *Heterotestophyes* throughout the globe, namely *H. sobolevi* (Leonov, 1957) reported in terns and gulls (*Sterna hirundo* and *Hydroprogone*) from Eurasia and Australasia and *H. neimongolis* (Qiu *et al.* 1984). The locality and host of *H. neimongolis* not available in Bray *et al.*, (2008). A single species *H. heckmanni* sp.n. recovered from same host little tern (*Sternula albifrons*) in Jamshoro, Pakistan, were reported earlier (Siyal *et al.*, 2016). The present species *Heterotestophyes gibsoni* are being reported for the first time from Sindh, Jamshoro, Pakistan.

MATERIAL AND METHODS

Fifteen bird's little tern (*Sternula albifrons*) belongs to family Sternidae were shot
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down from Jamshoro for helminth parasite infection at random intervals and brought to the parasitology laboratory, Department of Zoology, University of Sindh, Jamshoro, Pakistan. The birds were dissected and examined for collection of internal helminth parasites. During examination of gut contents and visceral organs five mature specimens were collected from small intestine of two birds. Later these specimens were fixed in hot steaming 70% ethanol, where trematodes expand and instantly die. The specimens were gently placed over clean glass slide, pressed lightly with another, tied with thread and fixed in formalin, acetic acid and 70% ethanol (F.A.A) prepared in a ratio of 6:2.5:100, for twenty-four hours, stained with Mayer's carmalum, dehydrated in graded series of ethanol, cleared in clove oil and rinsed with xylene. Finally, the specimens were permanently mounted in canada balsam for further study (Schmidt, 1992). Line drawings were prepared with the aid of a camera Lucida. Body measurements (length and width) are given in millimeters (mm). Photomicrographs were prepared with the courtesy of Vertebrate Pest Control Institute, Southern Zone Agricultural Research Center, Karachi University Campus, Karachi. Specimens were deposited in senior author's collection, Department of Zoology, University of Sindh, Jamshoro.

RESULTS

Heterotestophyes gibsoni sp.n.

Host: Little tern (*Sternula albifrons*)

Location: Small intestine

Locality: Jamshoro, Sindh, Pakistan

No. of hosts examined/ infected: 15/02

No. of specimens recovered: 05

Etymology: Species name refers to Dr. D.I. Gibson, Former head of Parasitology section, British museum, London.

Description is based upon five, stained, permanently mounted, egg bearing mature specimens.

Delicate worms, body small, divided into fore and hind body. Total body length 0.93-1.00 (0.976) by 0.3-0.32 (0.308) wide at the level of mid body region, some distance below the genital atrium and above the ovary, narrower in the anterior region, smaller and twisted at the level of pharynx and broadly rounded in the

posterior region. The body measurements of the reported species have been presented in Table I.

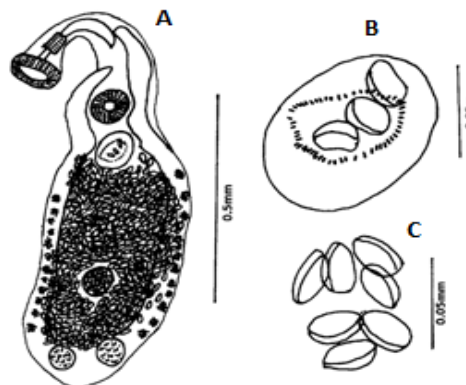


Figure 1: *Heterotestophyes gibsoni* sp.n. holotype, A) entire worm B) enlarged genital sac with spines C) eggs enlarged



Figure 2: *Heterotestophyes gibsoni* sp.n. entire worm, holotype photomicrograph (183x)

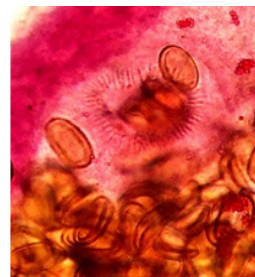


Figure 3: Enlarged genital sac with spines photomicrograph (198x)

Oral sucker terminal, rounded, highly muscular and broader than long, ranged from 0.05 to 0.06 (0.058) by 0.07-0.09 (0.082). Pre-

pharynx very short, hardly 0.01-0.01 (0.01). Pharynx well developed, muscular 0.02-0.03 (0.024) by 0.03-0.03 (0.03). Esophagus long 0.1-0.1 (0.1). Intestinal bifurcation is above the acetabulum, caeca long, obvious, caeca terminate in hind body. Distance between caecal

bifurcation and acetabulum is 0.1-0.1 (0.1). Ventral sucker muscular, rounded, occupy 2nd quarter of the body, closer to anterior extremity 0.06-0.07 (0.068) by 0.07-0.07 (0.075). The distance between oral and ventral sucker is 0.31-0.32 (0.316).

Table I: Comparative morphological features of species of the genus *Heterotestophyes* Leonov, 1957 recovered in Sindh, Pakistan and Eurasia and Australasia

Species	<i>Heterotestophyes sobolevi</i> (Leonov, 1957)	<i>Heterotestophyes neomonglis</i> (Qiu et al. 1984)	<i>Heterotestophyes heckmanni</i> sp.n. (Siyal et al., 2016)	<i>Heterotestophyes gibsonisp.n.</i> (present study)
Hosts	<i>Sterna hirundo</i> and <i>Hydroprogone</i>	-----	<i>Sternula albifrons</i>	<i>Sternula albifrons</i>
Locality	Eurassia and Australasia	-----	Jamshoro, Sindh, Pakistan.	Jamshoro, Sindh, Pakistan.
Location		Intestine	Intestine	Intestine
Body size	1.3-2.0		1.21 by 0.29	1.00 by 0.32
Oral sucker	With transverse ridge	With transverse ridge	Without transverse ridge	Without transverse ridge
Pre-pharynx	-----	Long	Very short, hardly 0.02	very short hardly 0.01
Pharynx	-----	Short	0.1 by 0.03	0.03 by 0.03
Esophagus	-----	Very short	Long 0.13	0.1 long
Intestinal bifurcation	-----	Bifurcates just below the pharynx, apart from acetabulum	Bifurcates above the acetabulum at distance above the acetabulum 0.05	Bifurcates at some distance below the pharynx, far from above the acetabulum at distance 0.1
Ventral sucker	-----	Situated in third quarter of the body	0.16 by 0.15 situated just above half of the body	0.07 by 0.07 occupy 2 nd . quarter of the body
Sucker ratio	-----	-----	0.75	0.85
Genital atrium size/location	-----	Occupy in third quarter of the body	0.12 by 0.13 occupy slightly below half of the body	0.07 by 0.09 occupy at middle of the body
Genital sac spines	70-120	39	47	57
Genital pore				Inside the genital sac, above the spines
Seminal vesicle	Bipartite	Bipartite	Oval 0.08- by 0.05	Not obvious
Ovary	-----		0.07 by 0.07 distance b/w ovary & anterior testis 0.08	0.06 by 0.06 distance b/w ovary & testes 0.1
Testes	-----	Testes tandem	Anterior testis: 0.07 by 0.05 Posterior testis: 0.071 by 0.05, tandem	Anterior testis: 0.06 by 0.06 Posterior testis: 0.05 by 0.05, juxta-opposite
Vitelline follicles	-----	Commence from below genital sac, extend up to base of anterior testis	commence form slightly above the ovary, arranged in lateral fields up to the base of posterior testis.	Commence from below genital sac, extend up to above the testes.
Eggs	24-32 by 14-20 um	-----	0.024-0.035 by 0.015-0.015.	0.02-0.03 by 0.15-0.02

Sucker ratio 0.83. Testes lie in posterior most region of the hind body, these are Juxta-opposite, rounded in shape, situated towards margin in lateral position. Anterior testis 0.05-0.06 (0.056) by 0.06-0.06 (0.06), posterior testis

is 0.04-0.05 (0.046) by 0.04-0.05 (0.046) in size. Distance between two testes is 0.03-0.04 (0.032). Genital atrium occupies middle of the body, elongated to rounded in shape, wider than long, distinctly separated from acetabulum, 0.07-

0.07 (0.07) by 0.09-0.09 (0.09). Spines in the crown are 57-60 in number. Genital opening inside the genital sac, above the spines, Seminal vesicle clearly not obvious due to profuse uterus. Ovary pre-testicular, rounded in shape, sub median, equal in size to anterior testis, 0.06-0.06 (0.06) by 0.06-0.06 (0.06). The distance between ovary and anterior testis is 0.99-0.1 (0.278). Uterus profuse with loops in hind body, the uterine loops are inter-caecal between the genital atrium and anterior testis. Vitellaria follicular, commence at little distance below the genital atrium, extend up to the hind body above the testes. Arms of excretory vesicle not obviously observed due to profuse uterus. Eggs are oval shaped, double walled 0.02-0.03 (0.024) by 0.15-0.02 (0.12).

DISCUSSION

The specimens recorded during the present study were recovered from the small intestine of little tern (*Sternula albifrons*), in Jamshoro, Sindh, Pakistan. According to the accessible literature two species of the genus *Heterotestphyes* (Leonov, 1957), belongs to the family Heterophyidae (Leiper, 1909), are known from avian hosts throughout the world.

Heterotestphyes sobolevi (Leonov, 1957) was reported interns and gulls (*Sterna hirundo* and *Hydroprogone*) from Eurasia and Australasia and *H. neimongolis* (Qiu et al. 1984). The locality and host of *H. neimongolis* not reported by Bray et al. (2008). The specimens recorded during the present study differ from *H. sobolevi* with respect to body size. In *H. sobolevi*, oral sucker was dorsal transvers muscular ridge, separated by parenchyma, circles of spines in the genital sac was 70-120, eggs was 24-32 by 14-20 μm , arms of excretory vesicle extend interiorly beyond ovary, while in present specimens, oral sucker was without transverse ridge, crown of spines are 57-60 in genital sac, eggs were 0.02-0.03 by 0.15-0.02 in size and arms of excretory vesicle were not obvious due to excessive uterus.

The specimen recorded during the present study was also different from *H. neomonglis* mainly in general body features. In *H. neomonglis* body of worm was straight, oral sucker larger with transverse ridge, long pre-pharynx, small esophagus, intestinal bifurcation just below the pharynx far from acetabulum, ventral sucker larger, genital sac smaller in size than acetabulum and oral sucker, crown of

spines are 39, both testes were oval, touching each other, while in present specimens oral sucker was smaller without transverse ridge, short pre-pharynx, long esophagus, intestine bifurcates at some distance below the pharynx, far above the acetabulum, distance between caecal bifurcation and acetabulum was comparatively less than *H. neomonglis*, genital sac was approximately same in size with acetabulum, both testes are juxta-opposite were rounded and occupy some distance in the hind body, uterine coils in *H. neomonglis* very few, genital opening occupies the same position in genital sac little above the spines.

The specimen recorded during the present study differ from *H. heckmanni* sp.n. (recovered during present study from the same host) in having smaller body size 1.00 by 0.32, while in *H. heckmanni* sp.n. the body size is 1.2 by 0.29. In present specimen, the anterior region was narrower, twisted at the level of pharynx and rounded in posterior region, while in *H. heckmanni* sp.n. the anterior region is narrower, little invagination at the level pharynx and the posterior region is roughly V shaped. In present specimens the oral sucker, pre-pharynx, pharynx, genital atrium, acetabulum, are smaller in size, while in *H. heckmanni* sp.n. oral sucker, pre-pharynx, pharynx, genital atrium, acetabulum, are larger in size. Intestinal bifurcation in present specimens was at some distance above the acetabulum, while in *H. heckmanni* sp.n. caecabifurcates above the acetabulum. The crown of spines in present specimens were 57-60, while in *H. heckmanni* sp.n. the crown of spines were 41-47. The ovary and testes were smaller in size, while in *H. heckmanni* sp.n. these were larger. In present specimens both testes were rounded, juxta-opposite, situated in lateral position in hind body and distance between testes and ovary is 0.99-0.1, while in *H. heckmanni* sp.n. the anterior testis occupies left margin in lateral position and posterior testis was situated just below the anterior testis in hind body and distance between anterior testis and ovary was 0.08-0.09. Vitellarium in present specimens start at little distance below the genital sac, exceed up to above the testes, while in *H. heckmanni* sp.n. it commences from above the ovary, along the lateral fields up to the base of posterior testis. In present specimens the uterine coils were inter-caecal, while in *H. heckmanni* sp.n. uterine loops were profuse, completely fill in the space in hind body and cover the caeca, terminating of caeca were not obvious due to eggs.

Main differences noted from reported species of the genus *Heterotestophyes* and *H. heckmanni* (Siyal *et al.*, 2016) were:

- (a) Size and shape of the body
- (b) Size of Pharynx
- (c) Position of caecal bi-furcation
- (d) Size of ventral sucker
- (e) Number of spines in genital sac
- (f) Arrangement of vitelline follicles.

Keeping in view the above mentioned specific differences, a new species was proposed *Heterotestophyes gibsoni* sp.n. The species name refers to Dr. D.I. Gibson, Former head of Parasitology section, British museum, London.

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