ECHINOSTOMA GARZETTI SP.N. (DIGENEA: ECHINOSTOMATIDAE) IN *EGRETTA GARZETTA* (LITTLE EGRET) IN SINDH, PAKISTAN.

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Abstract

The new species *Echinostoma garzetti*, is characterized by having smaller oral sucker, smaller pre-pharynx and esophagus. A number of collar spines (which are 47 in number), cirrus sac being kidney shaped with thick walls, testes bi-lobed, larger in size with rough indentations.

Introducation

The genus *Echinostoma* Rudolphi, 1809 is worldwide in distribution. It was erected to accommodate termatodes recovered from birds, fishes, reptiles and mammals. The genus *Echinostoma* is a well-established genus with numerous species reported from all most all over the world from Avian hosts. The genus *Echinostoma* belongs to the family Ehinostomatidae Loss, 1899 and sub Family Echinostomatinae Looss,1899.

Type species is *Echionostoma revolutum* (Froelich, 1802) looss,1899; *E. armatum* Molin, 1858; *E. revolutum* var. *japonicum* Kurisu, 1932; syn. *E. miyagawai* Ishii, 1932; *E. paraulum Dietz,* 1909 is recovered in various aquatic and terrestrial birds of the families including Anatidae, Phalacrocoracidae, Scolopacidae, Ardeidae, Raillidae, Phasianidae, Corvidae, Columbidae etc., occasionally in man. Loss, Tubangui, Sprehn, Yang, Beaver Bashikirova, Yamaguti etc: *E.echinatum* Loos (1899).

Eight species of the genus reported from avian hosts in Pakistan are: *E. lahorensis* Bhutta and Khan, 1974, recovered through experimental studies in day old chicks; *E. revolutum* (Froelich, 1802) Rudolphi, 1809, Bhutta and Khan, 1975 from *Anas Platyrhynchos*; *E. chloropodis philippinensis* (Tubangui, 1932) Bhutta and Khan, 1975 from *Gallinula chloropus*; *E. atrae* Birmani *et l*., 2008 from *Fulica atra*; *E. sindhenses* Dharejo *et al.*, 2009 from *Bubulcus ibis* and *E. megaovata* Dharejo, 2006 (Unpublished part of thesis) from *Fulica atra* respectively; *E. valentini* Sanjota and Ghazi, 2011 from *Phalacrocorax fusicolis* and *E. rafiae* Bushra *et al.*, 2014 from *Egretta garzetta*.

Material And Methods

The birds *Egretta garzetta* were shot down from Jamshoro District, Sindh, Pakistan. The birds were autopsied in the laboratory for collection of intestinal Helminth parasites. Single specimen of a trematode was recovered from the small intestine of a host. The specimen was thoroughly washed in saline solution to get rid of mucus. Later it was fixed in 70% ethnol. The specimen was gently placed over a clean glass slide, pressed lightly with another slide, tied with thread and placed in F.A.A. solution for twenty-four hours. The specimen was stained with Mayer's carmalum, dehydrated in graded series of alcohol, cleared in clove oil, rinsed with xylene and permanently mounted in preserva media. Drawings were prepared with the aid of a Camera Lucida. Measurements are given length by width in millimeters and photomicrograph was prepared through Olympus Digital microscope MIC-D at SARC/ PARC, Karachi University campus 75270. Specimen is deposited in senior author's collection, Department of Zoology, University of Sindh, Jamshoro.

***Echinostoma garzetti* sp. n.**

(Figs.1-5)

Host: *Egretta garzetta*
Locality: District, Hyderabad.
Site of infection: Small intestine
Number of hosts examined/infected: 02/ 01
Number of specimen recovered: 01
Etymology: Species name refers to the species of the host bird.

Description is based on single, mature, egg bearing, and permanently mounted specimen: Body is medium sized, much flattened and broadly rounded in posterior region, maximum width is attained in the posterior region of the body below the testes. Total body length is 10.06mm by 2.0mm in size. The cuticle is smooth without spines or other patterned structures.

Head collar is well developed measure 1.0 in size with dorsally uninterrupted crown of 47 spines.

The oral sucker is sub-terminal, muscular and smaller than the pharynx and ventral sucker. It measures 0.2mm by 0.21mm.

Pre-pharynx short, measure 0.11 by 0.05. Pharynx is well developed prominent, elongated and oval measure 0.3 by 0.2.

The esophagus is 0.25 by 0.09 in size, nearly reaches the acetabulum. Intestinal bifurcation is just in front of the ventral sucker. Intestinal ceaca simple and smooth extend to near the posterior end of the body.

The acetabulum lies in the first quarter of the body, larger than the oral sucker, rounded to elongated and muscular, measure 1.3 by 0.99 in size. Sucker ratio being 1:1.05.

The cirrus sac is well developed, rounded, roughly kidney shaped, with thick walls, measure 0.5 by 0.29 in size.

Metraterm well developed opens into a common genital opening. Genital opening below the ceacal bifurcation, on the anterior border of the acetabulum.

Ovary spherical, median pre-testicular, measure 0.5 by 0.52 in size, the distance between the first testis and ovary is 0.4.

The testes are in posterior half of the body, contiguous; elongated with rough indentations, bilobed with upper lobes broader than the lower lobes, the anterior testis measure 1.15 by 0.72 and the posterior testis is 1.46 by 0.8 in size.

Uterus short with few loops lie between ovary and ventral sucker.

The vitelline follicles lie in two lateral fields these commence from below the ventral sucker and proceed up to the posterior extremity. The vitelline follicles are separated by excretory vesicles in the posterior region.

Eggs are oval- shaped, double walled 0.09-0.012 by 0.05-0.06 in size.

Discussion

Present specimen *Echinostoma garzetti* is distinguished from the earlier reported species of the genus *Echinostoma* Rudolphi, 1809 recovered from avian hosts in Pakistan mainly in having following differentiating characters:

- Number of collar spines.
- Varying length of pharynx and esophagus.
- Different sucker ratio.
- Shape and size of ventral sucker.
- Shape and size of cirrus sac.
- Shape and size of ovary.
- Shape and size of testes.
- Less uterine loops.
- Larger egg size.

The body size in present specimen is 10.06 by 2.0. while body size: in *E. valentine* is 5.5-6.4 by 0.7-0.94; in *E. megaovata* is 5.8 by 0.95; in *E. sindhensis* it is 13.7 by 3.7; in *E. atrae* it is 14.21-15.90 by 1.50-1.64; *E. lahorensis* (not mentioned); *E. chloropodis*is 6.66-10.9 by 0.818; in *E. revolutum* the body size is 7.05 by 1.21 and in *E. rafiae* the body size is 10.2 by 2.5.

The present specimen closely resemble *E. rafiae* in having nearly equal body size, same avian host (Table-1), but differences are obvious in the number of collar spines which are 47 in present specimens and in *E. rafiae* these are 41 in number; the vitelline follicles commence from below the ventral sucker in present specimen, while in *E. rafiae* these start from mid of ventral sucker; the ovary in present specimen is spherical and smaller than testes while in *E. rafiae* the ovary is larger than testes, the testes in the present specimen are quite larger in size, contiguous, bilobed with slight indentations, the uterus has few loops and the eggs are quite larger in size (fig-1) while in *E. rafiae* the anterior testis is bilobed while the posterior testis is roughly irregular in shape, the uterus has moderate loops and eggs are smaller.

The host in *E. valentini* is *Phalacrocrax fusicolis* shot down at fish rearing farms, Thatta; *E. megaovata* and *E. atrae* are reported from *Fulica atra* from Hyderabad and Manchar lake; *E. lahorensis* is reported from day old chick (experimental studies) from Lahore; *E. chloropodis* reported from *Gallinula chloropus* from Balloki Head works; *E. revolutum* was recovered from *Anas platyrhynchos* from Balloki Head works.

The oral sucker in present specimen is 0.2 by 0.21, while in *E. sindhenses* it is larger in size being 0.9 by 0.3. In rest of the species mentioned above (Table-1) the oral sucker is near about the size of the oral sucker in present specimen

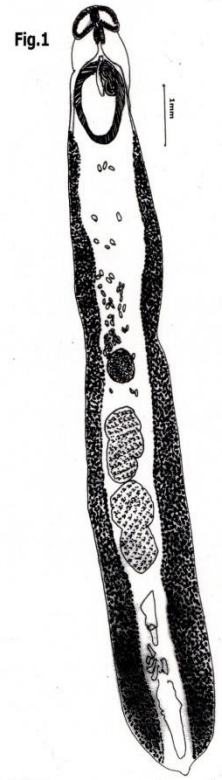
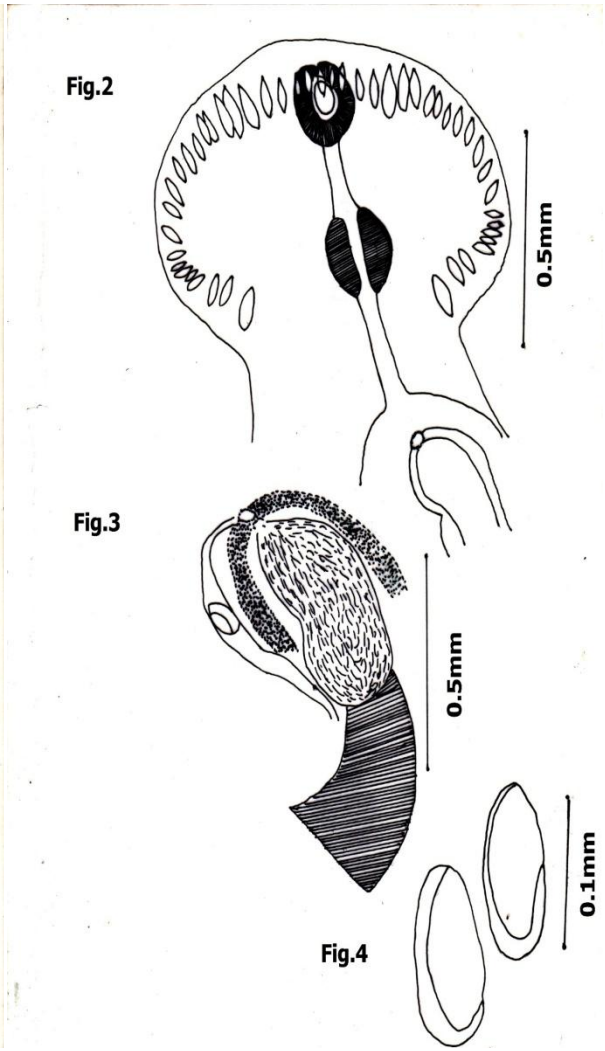


Fig.1 *Echinostoma garzetti* sp.n., Holotype, entire worm



Figs.2-4 *Echinostoma garzetti* sp. n.
Fig.2 Head collar spines enlarged with oral sucker, short prepharynx, muscular pharynx and part of esophagus.
Fig.3 Cirrus sac, Metaterm and post bifurcal genital opening.

Fig.5

Fig.5 *Echinostoma garzetti* sp.n.,entire worm, holotype. Photomicrograph (125X).

The number of collar spines are 47 in present specimen, while in *E. valentini* it is 35; *E. megaovata* 35; *E. sindhenses* 50; *E. atrae* 38; *E. lahorensis* it is 45; in *E. chloropodis* 46; *E. revolutum* 37 and in *E. rafiae* the collar spines are 41.

Pre-pharynx and pharynx in present specimen is 0.11 by 0.05 and 0.33 by 0.2. In *E. atrae* the pre-pharynx is hardly visible while the size of pharynx is nearly equal. In most of the species the pre-pharynx is absent or smaller in size.

Esophagus in present specimen is 0.24 by 0.09, while in *E. rafiae* and *E. revolutum* the esophagus is nearly equal, while in rest of the species the esophagus is quite larger in size (Table-1).

Ventral sucker in present species is 1.3 by 0.99 it resembles with *E. rafiae* in size and in rest of the species the ventral sucker is smaller in size.

Cirrus sac is kidney shaped 0.5 by 0.29 in size. It is equal in size with *E. rafiae* and it is oval shaped and in rest of the species the cirrus sac is smaller in size (Table-1)

Ovary in present specimen is 0.5 by 0.25, spherical and resemble in size with *E. sindhensis* and *E. atrae.*

Testes in present specimen are elongated, bilobed the anterior testes is 1.15 by 0.72 and posterior testes is 1.46 by 0.8. In *E. sindhensis* the anterior and posterior both testes are larger in size while in rest of the species the testes are smaller in size. (Table-1)

Eggs in present specimen are 0.09-0.12 by 0.05- 0.056 in size, these match with *E. megaovata and E. chloropodis*. Size of ova in rest of the species are smaller than the present specimen.

Table:1 Comparative body measurements of species of the genus *Echinostoma* reported in avian hosts in Pakistan.

Species	*E. lahorensis*, Bhutta and khan 1974	*E. revolutum*, (Froelich, 1802) Rud 1809., Bhutta and Khan 1975	*E. chloropodis* Bhutta and Khan, 1975	*E. atrae* Birmani., 2008	*E. megaovata* Dharejo, 2006 (Unpublished part of thesis)	*E. sindhenses* Dharejo *et al*., 2009	*E. valentini* Sanjota and Ghazii 2011	*E. rafiae* sp.n Bushra *et al*., 2014	*E. garzetti.* sp. n.
Hosts	Day old chick (experimental studies)	*Anas platyrhynchos*	*Gallinua chloropus*	*Fulica atra*	*Fulica atra*	*Bubulcus ibis*	*Phalacrocorax fusicolis*	*Egretta garzetta*	*Egretta garzetta*
Locality	Lahore	Balloki Head works	Balloki Head works	Manchar lake	Hyderabad	Hyderabad	Thatta	Hyderabad	Jamshoro
Body size		7.059by 1.121	6.66-10.9 by 0.818-1.332	14.21-15.90 by 1.50-1.64	5.8 by 0.95	13.7by 3.7	5.5-6.4 by 0.7-0.94	10.2by 2.5	10.06 By 2.0
Oral sucker		0.245by 0.245	0.215-0.225	0.17-0.28by0.25-0.50	0.20by0.31	0.9-0.30	0.12-0.13by0.16-0.18	0.3by0.3	0.2 by 0.21
Collar size					\			1.5	1.0
Collar spines	45	37	46	38	35	50	35	41	47
Prepharynx		indistinct	Absent		0.2L	Hardly visible	0.030-0.032	Present 0.05by0.05	Present 0.11by0.05
Pharynx		0.215by0.186	0.176-0245by0.127-0.186	0.31-0.35 by 0.21-0.32	0.29by0.20	0.10-0.14	0.15-0.17by0.07-0.09	0.20by0.2	0.3 by 0.2
Esophagus		0.215by0.186	0.333-0.454	0.35-0.51	1.1	0.55-0.79	0.56-0.59 by0.04-0.045	0.29by0.1	0.25 by 0.09
Ventral sucker		0.939by0.757	0.757-0.909 by0.606-0.909	1.35-1.57by1.20-1.28	0.38by0.39	0.11-0.20by0.13-0.15	0.72-0.91 by0.57-0.72	1.2by1.1	1.3 by 0.99
Gential opening	Post bifurcal	Post bifurcal	Post bifurcal	Post bifurcal	Post bifurcal	Post bifurcal	Post bifurcal	Post bifurcal	Post bifurcal
Cirrus sac		0.454		0.20by0.51	0.29by0.12	0.13-0.15by 0.19-0.2	0.31-0.33by 0.43-0.63	Oval 0.52by0.24	Kidney like 0.5 by 0.29
Vitelline follicles	Commence from below the ventral sucker	Commence from below the ventral sucker	Commence from below the ventral sucker	Commence from below the ventral sucker	Commence from below the ventral sucker	Commence from below the ventral sucker	Commence from below the ventral sucker	Start from mid of ventral sucker	Commence from below the ventral sucker
Ovary		0.294by0.294	0.264-0.454 by0.264-0.424	0.50-0.57 by0.42-0.46	0.20by0.25	0.5-0.6by 0.8-0.9	0.26-0.28 by0.62-0.28	0.8by0.8	.0.5 by 0.52
Testes		Ant: 0.441by 0.294 Post: 0.548by0.294	Ant: 0.606-0.909by0.212-0.363 Post: 0.606-0.818by0.42-0.363	Ant: 1.07-1.77by 0.28-0.40 Post: 0.40-1.14 by0.28-0.37	Ant: 0.35by0..23 Post: 0.32by0.25	Ant: 1.231by0.970 Post: 1.94by0.820	Ant 0.47-0.65 by0.43-0.63 Post: 0.56-0.71 by 0.43-0.63	Ant: 0.65by0.67 Post: 0.59by0.79	Ant 1.15 - 0.72 Post 1.46-0.8 Bilobed
Uterus		Moderate loops	Moderate loops		Few loops	Few loops	Few loops	Moderate loops	Few loops
Eggs		0.63-0.089	0.104-0.107by0.071-0.76	10-15by11-86	100-115by5o-70	87.5-105by62.5-70	0.062by0.04 0.072by0.04	0.062by0.04 0.072by0.04	0.09-0.012 by o.o5 by 0.056

As the present specimen do not match exactly with rest of the species mentioned above particularly, in number of collar spines, shape and size of cirrus sac, the size and shape of gonads, the size of eggs which are larger than the other species except *E. megaovata* and *E. chloropodis*, differ from species reported in Pakistan. A new species *E. garzetti* is proposed. The species name refers to the host species.

Ackowledgment

Authors wish to thank Mr. Muzfar Ahmed Senior scientific officer Pakistan Agricultural Research center (PARC) Southern zone Agricultural center SARC, University of Karachi 75270, for providing digital Olympus MICD camera for photography of specimens.

Authors also wish to extend their gratitude to Dr Noor-un- for Senior scientific officer, Pakistan Agricultural Research center (PARC), Southern zone Agricultural center SARC, University of Karachi 75270, access to the literature and for his moral help.

Authors also wish to extend their thanks to the Medical Zoology Laboratory staff for their help and support in the autopsy examination of birds and preparation of Parasites for study.

REFERENCES

Bhutta, M.S. and Khan, D. (1974). Studies on the life cycle of *Echinostoma lahorensis*, new species (Trematoda: Echinostomatidae) Pakistan *J. Zool.,* 6: pp. 123-139.

Bhutta, M. S. and Khan, D. (1975). Digenetic trematodes of vertebrates from Pakistan. Bullet in Department of Zoology University of Punjab, (N.S.), article 8, pp. 1-175.

Birmani N. A., Dharejo, A. M. and Khan, M. M. (2008). *Echinostoma atrae*, new species (Digenea: Echinostomatidae) in Black Coot *Fulica atra* (Aves: Rallidae) of Manchar Lake, Sindh, Pakistan. Pakistan. *J. Zool*., 40: 379-383.

Bushra, S., Sanjota, N.D., Abbasi, M.A.A. and Khan, A. (2014). *Echinostoma rafiae* new (Trematoda: Echinostomatidae) from the Little egret *Egretta garzetta* in Sindh, Pakistan. Int. *J. Biol. Biotech*., 11 (4): 491-495.

Dharejo, A.M. (2006). Trematode parasites of birds of different feeding habits of Hyderabad District, Hyderabad, Sindh, Pakistan. Ph.D. thesis, Faculty of Natural Sciences, University of Sindh, Jamshoro, Pakistan.

Dharejo, A.M., Bilqees, F.M. and Khan, M.M. (2009). *Echinostoma sindhenses* new species (Digenea: Echinostomatidae) from Cattle Egret *Bubulcus ibis* (Ardeidae) of Hyderabad, Sindh, Pakistan. *Proc. Parasitol*., 48: 73-80.

Dietz, E. (1909). Die Echinostomiden der Vogel. Zool. Anz. 34: 180-192.

Froelich, J. A. (1802). Beiträge zur Naturgeschichte der Eingeweiderwurmer. Naturf., Halle, 29: 5-96.

Ishii N. (1932). Studies on bird trematodes. I. Bird trematodes in Japan. II. Four new species. Jap. J. Exp. Md., 11: 91-100.

Jones, A. Bray, R. A. and Gibson D. I. (2005). Keys to the Trematoda volume 2.The Natural History Museum, London. UK. CABI, Publishing. Wallingford. Oxfordshire., pp. 745.

Kurisu, Y. (1932). Studies an trematoden, Die in Japanischen Haushunnern Schmarotzen. J. Kunamoto Med. Soc 8: 283-297.

Looss, A. (1899). Weitere Beitrage zur kenntnis der Trematodenfauna Aegyptens, Zugleich Versuch einer naturlichen Gliederung des Genus *Distomum*Retzius. Zoologische jahrbucher. Abteilung fur Systematik, Geographie und Biologie der Tiere, 12: 521-784.

Molin, R. (1858). Prospectus helminthum, quae in Prodromo faunae helminthologicae venetae Continentur. Sitz-ber. Akad Wien math. Naturw. Class. Abt. 1. 30: 127-158.

Rudolphi, C. A, (1809). Entozoorum sive vermium intestinalium historia naturalis, Vol, 2, Part 1. 457pp. Amstelaedami.

Sanjota N. D. and Ghazi, R. R. (2011). *Echinostoma valentini* sp.n.(Trematoda: Echinostomtidae) from a bird *Phalacrocorax fuscicollis* in Sindh, Pakistan. Proc. Parasitol., 51: pp 87-97.

Shafi, M. M., Rehana. R., Ali, R. and Nisa, U. N., (1986). *Echinostoma bengalensis* sp. n. (Trematoda: Echinostomatidae) in Rice-Rat *Bandicota bengalensis* of Sindh, Pakistan. Proc. Parasit., 2: 28-35.

Tubangui, M. A. (1932). Trematode parasite of Philippine vertebrates. V. Flukes from birds. Philipp. J. Sci. 47: 369-404.

Yamaguti, S. (1958). Systema Helminthum Part I and Part II. Digenetic Trematodes of Vertebrates. Interscience. Publishers, New York.1575 pp.