

ECHINOSTOMA RAFIAE NEW SPECIES (TREMATODA: ECHINOSTOMATIDAE) FROM THE LITTLE EGRET EGRETTA GARZETTA IN SINDH, PAKISTAN

S. Bushra¹, N. Das Sanjota¹, M.A.A. Abbasi² and Aly Khan³

¹Department of Zoology, University of Sindh, Jamshoro, Sindh, Pakistan

²Centre for Environmental Sciences, University of Sindh, Jamshoro-76080, Pakistan

³Crop Diseases Research, Institute (CDRI), University of Karachi Campus, Karachi – 75270, Pakistan

Email Address: drsanjota82@gmail.com

ABSTRACT

During an investigation on helminth parasites of birds, a new species of trematode genus *Echinostoma rafiae* sp. n was recorded from the intestine of *Egretta garzetta* collected from Hyderabad, Sindh, Pakistan. The new species is characterized by having: forty one number of collar spines. Short Prepharynx, deeply indented testes, and larger spherical ovary.

Key-words: *Echinostoma rafiae* sp. n, *Egretta garzetta*, Hyderabad, Sindh, Pakistan.

INTRODUCTION

The genus *Echinostoma* Rudolphi, 1809 is worldwide in distribution and was erected to accommodate trematodes recovered from, fishes, reptiles, birds and mammals. The family Echinostomatidae Looss, 1809, is characterized by the presence of a circum oral head collar armed with one or two crowns of large spines interrupted ventrally, it embraces a broad range of final vertebrate hosts and wide geographical distribution.

The morphological diversity of this group have resulted in its sub divisions in to 21 nominal subfamilies and 91 nominal genera. (Jones *et al*; 2005). Accessible literature indicates over hundred species of the genus *Echinostoma* from avian hosts all over the globe.

Seven 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. revolution* (Froelich, 1802) Rudolphi, 1809, Bhutta and Khan, 1975 from *Anas Platyrhynchos*; *E. chloropodis philippinensis* (Tubangui, 1932) Bhutta and Khan, 1975 from *Gallinula chloropus*; *E. sindhenses* and *E. megaovata* from *Bubulcus ibis* and *Fulica atrea* respectively (Dharejo *et al.*, 2009); *E. valentini* Sanjota and Ghazi, 2011 from *Phalacrocorax fuscicollis*. *E. bengalensis* Shafi *et al.*, 1986 was however, reported from the small intestine of the rice rat *Bandicota bengalensis* in Sindh, Pakistan.

MATERIAL AND METHOD

The bird *Egretta garzetta* was shot down from Hyderabad District Sindh, Pakistan. It was brought to the laboratory; viscera were taken out for detailed examination for helminth parasitic infections. single specimen of the trematode was recovered from small intestine of the host. The specimen was fixed in hot 70% alcohol, it was later tied under slide pressure of cover glass and fixed in F.A.A. solution (formalin, acetic acid and 70% alcohol), for 24 hours. The specimen was stained with Mayer's carmalum, dehydrated in graded alcohol, cleared in clove oil, rinsed with xylene and mounted permanently in Canada balsam. Drawings were prepared with the aid of camera lucida and photomicrograph was prepared through Olympus Digital micro scope MIC-D at SARC/ PARC, Karachi University campus. 75270. Measurements are in millimeters. Specimen is deposited in senior author's collection, Department of Zoology, University of Sindh, Jamshoro.

RESULTS

Echinostoma rafiae sp. n
(Figs. 1-5)

Host:	<i>Egretta garzetta</i>
Locality:	District, Hyderabad
Site of infection:	Small intestine
Number of hosts examined/infected:	02/ 01
Number of specimen recovered:	01

Description is based on single, mature, egg bearing, permanently mounted specimen:

Body fairly elongate, medium 10.2 by 2.5, narrower in the anterior region and broadly rounded in the posterior region, maximum width is attained in the posterior half of the body at the level of testes, fore body is very short, hind body dorsoventrally flattened. Tegument is ornamented with spines in the anterior neck region up to some distance in the post bifurcal acetabular region.

The collar is well developed, reniform with two distinct ventral lappets, each bearing a group of six comparatively smaller spines 0.03 by 0.01 in size, accompanied by four pairs of postero lateral spines, slightly larger 0.04 by 0.02 in size., these are further accompanied by ten pair of slightly more larger spines 0.06 by 0.03 in size, a single dorso-medial spine measures 0.07 by 0.03 in size. Total collar spines are forty one.

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

Species	<i>E. vallentini</i> Sanjota and Ghazii 2011	<i>E. atrae</i> Birmani., 2008	<i>E. megaovata</i> Dharejo, 2009	<i>E. sindhenses</i> Dharejo, 2009	<i>E. chloropodis</i> Bhutta and Khan, 1975	<i>E. revolutum</i> , (froelich, 1802) Rud 1809., bhutta and Khan 1975	<i>E. lahorensis</i> , Bhutta and Khan 1974	<i>E. raftae</i> sp.n
Hosts	<i>Phalacroco fuscicolis</i>	<i>Fulica atra</i>	<i>Fulica atra</i>	<i>Bubulcus ibis</i>	<i>Gallinua chloropus</i>	<i>Anas platyrhynchus</i>	Day old chick (experimental studies)	<i>Egretta garzetta</i>
Locality	Thatta	Manchar lake	Hyderabad	Hyderabad	Balloki Head works	Balloki Head works	Lahore	Hyderabad
Body size	5.5-6.4 by 0.7-0.94	14.21-15.90 by 1.50-1.64	5.8 by 0.95	13.7 by 3.7	6.66-10.9 by 0.818-1.332	7.059 by 1.121		10.2 by 2.5
Oral sucker	0.12-0.13 by 0.16-0.18	0.17-0.28 by 0.25-0.50	0.20 by 0.31	0.9-0.30	0.215-0.225	0.245 by 0.245		0.3 by 0.3
Collar size								1.5
Collar spines	35	38	35	50	46	37	45	41
Prepharynx	0.030-0.032		0.2L	Hardly visible	absent	Is indistinct		0.05 by 0.05
Pharynx	0.15-0.17 by 0.07-0.09	0.31-0.35 by 0.21-0.32	0.29 by 0.20	0.10-0.14	0.176-0.245 by 0.127-0.186	0.215 by 0.186		0.20 by 0.2
Esophagus	0.56-0.59 by 0.04-0.045	0.35-0.51	1.1	0.55-0.79	0.333-0.454	0.215 by 0.186		0.29 by 0.1
Ventral sucker	0.72-0.91 by 0.57-0.72	1.35-1.57 by 1.20-1.28	0.38by0.39	0.11-0.20 by 0.13-0.15	0.757-0.909 by 0.606-0.909	0.939 by 0.757		1.2 by 1.1
Genital opening	Post bifurcal	Post bifurcal	Post bifurcal	Post bifurcal	Post bifurcal	Post bifurcal	Post bifurcal	Post bifurcal
Cirrus sac	0.31-0.33by 0.43-0.63	0.20by0.51	0.29 by 0.12	0.13-0.15 by 0.19-0.2		0.454		0.52 by 0.24
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
Ovary	0.26-0.28 by 0.62-0.28	0.50-0.57 by 0.42-0.46	0.20 by 0.25	0.5-0.6 by 0.8-0.9	0.264-0.454 by 0.264-0.424	0.294 by 0.294		0.8 by 0.8
Testes	Ant 0.47-0.65 by 0.43-0.63 Post: 0.56-0.71 by 0.43-0.63	Ant:1.07-1.77 by 0.28-0.40 Post:0.40-1.14 by 0.28-0.37	Ant:0.35 by 0.23 Post:0.32 by 0.25	Ant:1.231 by 0.970 Post:1.94 by 0.820	Ant: 0.606-0.909 by 0.212-0.363 Post: 0.606-0.818 by 0.42-0.363	Ant: 0.441 by 0.294 Post: 0.548 by 0.294		Ant :0.65 by 0.67 Post: 0.59 by 0.79
Uterus	Few loops		Few loops	Few loops	Moderate loops	Moderate loops		Moderate loops
Eggs	0.062 by 0.04 0.072 by 0.04	10-15 by 11-86	100-115by 50-70	87.5-105 by 62.5-70	0.104-0.107 by 0.071-0.76	0.63-0.089		0.062 by 0.04 0.072 by 0.04



Fig. 1 *Echinostoma rafiae* sp.n.,
Holotype, entire worm.
Scale bar: 0.1mm



Fig. 5. *Echinostoma rafiae* sp.n
entire Holotype.

Fig. 2

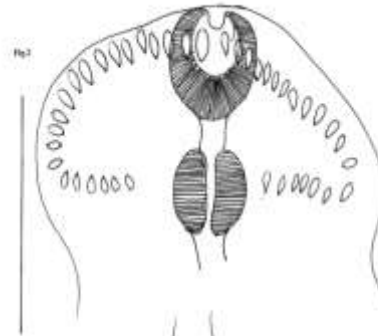


Fig.3

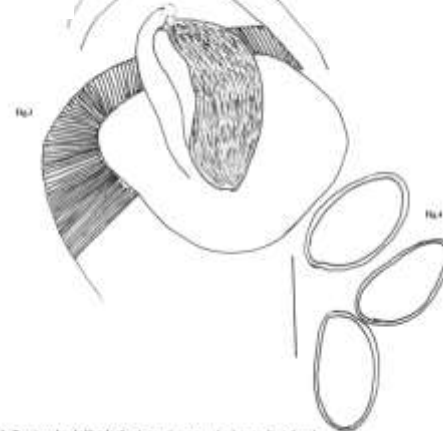


Fig. 4

Figs. 2-4 *Echinostoma rafiae* 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.4. Eggs enlarged.
Scale bar: 0.5 mm (Fig.2, 3); Scale bar: 0.05 mm (Fig.4)

Oral sucker terminal, smaller than the ventral sucker 0.3 by 0.3 in size. Prepharynx short 0.05 by 0.05. Pharynx elongated and oval 0.20 by 0.2. Esophagus fairly long 0.29 by 0.1. Distance between oral and ventral suckers is 0.61. The intestinal bifurcation is in the fore body and ceca reach closer to the posterior extremity.

Ventral sucker larger, muscular deeply cup shaped, closer to the anterior extremity 1.2 by 1.1. Sucker ratio being 1: 0.4.

Testes two tandem lie in the post, equatorial region, these have roughly indentation shape, or longitudinally elongated. The anterior testis is 0.62 by 0.62; posterior testis is 0.6 by 0.7.

The cirrus sac is small, pyriform in shape, lie in the fore body, anterodorsal to the ventral sucker, 0.52 by 0.24 in size. The genital pore lies just post bifurcal, above the acetabulum.

Ovary larger, anterior to testes, spherical in shape 0.7 by 0.7 in size.

The vitelline follicles lie in two lateral fields, these start at the level of mid ventral sucker and proceed up to the posterior tip of the body. The vitelline follicles do not fill in the space behind the testes, excretory vesicles is y shaped.

Uterus between ventral sucker and ovary with moderate number of loops. The uterine loops are intercaecal. Eggs are oval, elongate with smooth walls these are 0.062 - 0.07 by 0.04 - 0.04.

DISCUSSION

Family Echinostomatidae Poche, 1926 is diversified group with trematodes parasitizing fishes, Reptiles, Birds and mammals Yamaguti, 1958.

Literature indicates *Echinostoma* infection in avian hosts all over the globe.

More than hundred species of the genus *Echinostoma* Rudolphi, 1809 are reported from avian hosts. Those reported in Pakistan are: *E. lahorensis* Bhutta and Khan, 1974 recovered through experimental studies of life cycle in day old chick in Lahore, Punjab; *E. revolutum* (Froelich, 1802) Rudolphi, 1809; Bhutta and Khan, 1975 recovered in *Anas platyrhynchos* at Balloki Head works area, Punjab; *E. chloropodis philippinensis* (Tubangui, 1932) Bhutta and Khan, 1975 was recovered in *Gallinula chloropus* at Bulloki Head works in Punjab; *E.indhenses* and *E. megaovata* Dharejo *et al.*, 2009 were recovered from *Bubulcus ibis* and *Fulica atra* respectively in Sindh, *E. atrae Birmani et al.*; 2008 was recovered from *Fulica atra* of Manchar lake Sindh and *E. valentini* Sanjota and Ghazi, 2011 was recovered from *Phalacrocorax fuscicollis* in Sindh, Pakistan.

Following characters used for identification are considered significant at generic level:

The morphology of collar; number, shape, arrangement and relative sizes of collar spines; the morphology of male terminal genitalia, position of cirrus sac, structure of internal seminal vesicle, development of pars prostatica and size and armament of cirrus, position of the ovary and testes, location and structure of the vitellarium, the character of tegumental armament and the presence of a uroproct. Some additional characters such as: the size and shape of the body and length of the fore body, uterine and post testicular fields have also been applied. Jones *et al.* (2005).

Species identification is based upon: body size smaller if 5mm or less than 5mm long, medium 5 to 10mm and larger if longer than 10mm. collar number and size of collar spines, size of oral sucker, pharynx and esophagus, size, shape and position of gonads, size, shape and position of terminal genitalia, position of Vitellaria and sizes of eggs. Jones *et al.* (2005).

Present specimen is reported from *Egretta garzetta* while *E. valentini* is reported from a piscivorous bird *Phalacrocorax fuscicollis*; *E. atrae* and *E. megaovata* are reported from *Fulica atra*; *E.indhenses* is from *Bubulcus ibis*; *E. chloropodis* is reported from *Gallinula chloropus*, while *E. revolutum* is reported from *Anas platyrhynchos* and *E. lahorensis* is reported from a day old chick in experimental studies.

Present specimen is medium sized being 10.2 by 2.5 while *E. valentini*; *E. megaovata*; *E. chloropodis* and *E. revolutum* are smaller in size and *E. atrae* and *E.indhenses* are larger than the present species.

The number of collar spines are total 41 in present species while it is 35 in *E. valentini*, in *E. atrae* 38, in *E. megaovata* 35 and *E.indhenses* is 50, in *E. chloropodis* the number of collar spines are 46-47 and in *E. revolutum* the collar spines are 37 and in *E. lahorensis* it is 45.

The esophagus in present species is 0.29 by 0.1 while in *E. atrae*; *E. revolutum*; *E. chloropodis*; it is smaller in size and in *E. valentini*; *E. megaovata*; *E.indhenses* is larger in size.

In present specimen the ventral sucker is 1.2 by 1.1 while in *E. valentini*; *E. megaovata*; *E.indhenses*; *E. chloropodis*; *E. revolutum* it is smaller in size and ventral sucker in *E. atrae* is bit larger in size.

Genital opening lie post-bifurcal in all species including present specimen.

Cirrus sac in present specimen is 0.52 by 0.24 but while in all other specimens cirrus sac is smaller than the present specimen.

In present species the vitelline follicles start from the mid of ventral sucker while in all other species vitelline follicles commence from below the ventral sucker.

In present species the testes are with deep indentation and measure 0.65 by 0.67 (anterior), 0.5 by 0.79 (posterior). While in *E. valentini* the testes are heart shaped and smaller in size, in *E. atrae* the testes are elongated, slightly indented and larger, in *E. megaovata* the testes are subspherical with smooth walls and are smaller and in *E.indhenses* these are slightly lobed, larger. In *E. chloropodis* the testes are elongated, slightly indented and larger and in *E. revolutum* testes are longitudinally elongated with smooth walls and smaller in size.

The ovary in all the reported species are roughly rounded and smaller than the testes, in present species the ovary is larger than the testes and measure 0.8 by 0.8

Uterus has moderate loops in present species while in *E. valentini* it has comparatively few loops. In *E. atrae* the uterus has numerous loops, in *E. megaovata* and *E.indhenses* the uterus has few loops, in *E. chloropodis* and *E. revolutum* the uterine loops are moderate in number.

Eggs are smaller in present specimen, oval, elongated, measure 0.062- 0.07 by 0.04-0.04 in size. While in *E. valentini*; *E. chloropodis* and *E. revolutum* eggs are smaller in size while in *E. atrae*; *E. megaovata*; *E.indhenses* eggs are larger in size.

Present species is characterized by having: 41 collar spines, short Prepharynx, deeply indented testes, and larger spherical ovary, first recorded from *Egretta garzetta*, which has not been recorded in earlier reported species in Pakistan. Therefore, a new species *E. rafiae* is proposed. The species name is in honor of Dr. Rafia Rehana Ghazi (Retd.) Principle Scientific Officer and Director Vertebrate Pest Control Institute (SARC) PARC Karachi.

ACKNOWLEDGMENT

We would like to extend our graded thanks to Higher Education commission (HEC) for providing the funds for the research. Authors wish to thanks 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 wish to thanks Mohammad Saleem Unar Lecturer, Department of Zoology GBDC, GB, Thatta, and Mukhtiar Ahmed Channa Entomologist Sanghar for their help in shooting the birds from District Hyderabad.

REFERENCES

- Bhutta, M.S. and D. Khan (1974). Studies on the life cycle of *Echinostoma lahorensis*, new species (Trematoda: Echinostomatidae). *Pakistan J. Zool.*, 6: 123-139.
- Bhutta, M. S. and D. Khan (1975). Digenetic trematodes of vertebrates from Pakistan. *Bulletin Department of Zoology University of Punjab*, (N.S.), pp. 1-175.
- Birman N. A., A. M. Dharejo and M. M. Khan (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.
- 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., F.M. Bilqees and M.M. Khan (2009). *Echinostoma sindhenses* new species (Digenea: Echinostomatidae) from Cattle Egret *Bubulcus ibis* (Ardeidae) of Hyderabad, Sindh, Pakistan. *Proc. Parasitol.*, 48: 73-80.
- Froelich, J. A. (1802). Beiträge zur Naturgeschichte der Eingeweiderwurmer. *Naturf., Halle*, 29: 5-96.
- Jones, A. R. A. Bray and D. I. Gibson (2005). *Keys to the Trematoda*. volume 2. The Natural History Museum, London. UK. CABI, Publishing. Wallingford. Oxfordshire., pp. 745.
- Looss, A. (1899). Weitere Beitrage zur kenntnis der Trematodenfauna Aegyptens, Zugleich Versuch einer natürlichen Gliederung des Genus *Distomum* Retzius. *Zoologische jahrbucher. Abteilung fur Systematik, Geographie und Biologie der Tiere*, 12: 521-784.
- Poche, F. (1926). Das system der Platodaria. *Arch. Naturg. A*. 91: 458pp.
- Rudolphi, C. A. (1809). *Entozoorum sive vermium intestinalium historia naturalis*, Vol, 2, Part 1. 457pp. Amstelaedami.
- Sanjota N. D. and R.R. Ghazi (2011). *Echinostoma valentini* sp.n.(Trematoda: Echinostomidae) from a bird *Phalacrocorax fuscicollis* in Sindh, Pakistan. *Proc. Parasitol.*, 51: 87-97.
- Shafi, M. M., R. Rehana. R. Ali and U. N. Nisa (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.

(Accepted for publication August 2014)