Journal on New Biological Reports
JNBR 5(3) 122 – 128 (2016)

ISSN 2319 – 1104 (Online)

Published by
www.researchtrend.net

Description of a new species *Clinostomum awadhi* n.sp. (Trematoda: Clinostomidae) in *Phalacorcorax niger* (Aves: Phalacrocoracidae) of Sanghar, Sindh, Pakistan

Muhammad Moosa Abro, Ali Murtaza Dharejo, Muhammad Munif Khan and Nadir Ali Birmani

Government College Hyderabad Postgraduate Center, Hyderabad Sindh Pakistan.
Department of Zoology, University of Sindh, Jamshoro, Pakistan

***Corresponding author**: abromusa@gmail.com

| **Received**: 27 August 2016 | **Accepted**: 03 October 2016 |

ABSTRACT

The purpose of this paper is to present findings about new species of genus *Clinostomum* which was collected during helminthic study of Little Cormorant, *Phalacrocorax niger* of Sanghar Sindh Pakistan. A total of 46 Clinostomes were collected from the esophagus and gizzard of four hosts (Little Cormorant, *Phalacrocorax niger*). Present specimens have elongated tongue-shaped body, prominent sucker, well developed ceca, ovary, testes and uterus. Present new species further differs from its congeners in body shape, distribution of vitellaria, shape and position of testes, ovary and cirrus sac and uterus. On the basis of these differentiating characteristics, the present species is identified as a new species and named as *Clinostomum awadhi*. Species name refers to the locality of host bird.

Key Words: Avian trematode, *Clinostomum awadhi*, new species, *Phalacorcorax niger*, Sindh, Pakistan.

INTRODUCTION

The genus *Clinostomum* is common parasite of bird, fishes and snails. It uses snails, fishes and rarely frogs and toads as their intermediate hosts (Bullard & Overstreet, 2008, Lo CF et al. 1982 & Olsen, 1974). The adult forms are found in mouth, esophagus, oral cavity, pharynx or esophagus of fish eating birds, reptiles and occasionally mammals, including man (Gustinelli et al. 2010). The fish eating birds harbour adult *Clinostomum* include; *Ardea*, *Ardeola*, *Cancroma*, *Mycteria*, *Egretta*, *Nycticorax*, *Pelecanus*, *Nyctanass*, *Larus*, *Butorides*, *Phalacrocorax*, *Bubulcus*, *Botaurus*, *Plegadis*, *Gallinula*, *Podiceps*, *Ixobrychus*, *Sterna*, *Anhinga Herodias* and *Jabiru* etc (Yamaguti, 1971). These flukes are reported in North, Central and South America, Europe, Asia, Australia and Africa (Bullard & Overstreet, 2008 and Thatcher, 1981). A few species of genus *Clinostomum* cause yellow grub disease in fishes which result in mortality of fishes in various parts of world (Paperna, 1996, Szalai & Dick, 1988 and Lo CF et al. 1982). It makes fishes unsuitable for human diet. People get infected by consuming raw or half-cooked infected fish and results in Clinostomiasis (Halzoun disease) (Chung et al. 1995, Eiras, 1994, Yoshimura, et al. 1991 & Szalai & Dick, 1988). It also causes eye infection (Tiewchaloern, 1999). Moreover, birds being definitive host bear great pathological effect on the oral and esophageal epithelium, as acute inflammation in mucosal and submucosal layers and occasionally in muscular layer (Sutili et al. 2014). Host under study

Phalacrocorax niger is a Piscivorous in habit and forage singly or in loose group in ponds, lakes, streams and coastal areas and is migratory as well as resident bird (Zeenath, 2009, Sarker & Naher, 2002 & Roberts, 1991) have high potential to carry this parasite. *Phalacrocorax niger* is commonly found in District Sanghar Sindh, Pakistan (Rais et al. 2011). Moreover, reports are available on helminth parasites of *Phalacrocorax niger* in Pakistan includes Akram, 1996, & Dharejo et al. 2010. Bhutta and Khan, 1975 reported *Clinostomum singhi* from birds of Peshawar. Previously Abro et al. 2016a, 2016b, 2016c, 2016d & 2016e) recorded *Clinostomum complanatum*, *Macrobilharzia macrobilharzia*, *Paryphostomum sanghari*, *Euclinostomum heterostomum* and *Paryphostomum* from the same host birds in Pakistan. However, this is second report of genus *Clinostomum* in *Phalacrocorax niger* of Pakistan.

MATERIAL AND METHODS

Phalacrocorax nigers were brought alive from District Sanghar of Sindh Pakistan during December 2015 in Parasitology laboratory of Department of Zoology, University of Sindh, Jamshoro. The identification of hosts was made with help of description mentioned by Zeenath, 2009, Sarkar 2002 and Roberts, 1991. A total of eleven host birds were anthesized and dissected. The visceral organs including esophagus, gizzard, intestine, liver, heart and lungs were separated and kept in different petri dishes in normal saline. These organs were teased gently with needle. Samples were examined on stereomicroscope for the presence of helminth parasites. The collected trematodes were passed and fixed in 70% ethanol and pressed for overnight, stained with borax carmine, gradually dehydrated in alcohol series, cleared in clove oil and xylol. They were permanently mounted in Canada balsam. Camera Lucida was used to make drawing line and photograph was taken with Nikon digital camera. The measurements were taken in millimeter (mm) otherwise unit is stated properly. The identification of specimens was made accordance to keys given by Gibson et al. 2002, Yamaguti, 1971 and relevant literature.

RESULTS

During present study eleven Little Cormorants were examined and four were infected with 46 *Clinostomum* specimens. These were recovered from esophagus and gizzard of hosts and their infection rate was 36.36%. The description of specimens is given below.

Description (measurement is based on 20 specimens; min-max (average) ± standard deviation in millimeter scale):

Body of fluke is tongue shaped, elongated, measuring 8-10 (8.93) ± 0.694 long, 2.7-3.33 (3.003) ± 0.199 wide; attended at mid body. Anterior and posterior ends are slightly tapering. However, anterior end is slightly wider than posterior end. Oral sucker is well developed, subterminal, smaller than ventral sucker and measuring 0.65-0.86 (0.75) ± 0.071 long, 0.73-0.933 (0.83) ± 0.073. Ventral sucker is spherical and 0.97-1.0 **(**0.981) ± 0.0184 in diameter. Pharynx absent. Esophagus short, bifurcated into ceca in between ventral sucker and oral sucker. Ceca long, bifurcated, situated laterally in zigzag manner, extended up to posterior extremity.

Testes are prominent, slightly lobed, irregular shaped, unequal, lodged in second half of hindbody. They are separated by broad uterus. Anterior testis lies laterally to ovary and upper side of uterus. Anterior testis is 0.85-0.933(0.887) ± 0.027 long, 0.70-0.80 (0.747) ± 0.0355 wide. Posterior testis occupies entire intercecal area below uterus and measuring 0.35- 0.46 (0.4) ± 0.037 long, 0.95-1.20 (1.051) ± 0.092 wide. Uterus is broad, irregular, located in between posterior and anterior testes and measuring 1.01-1.55 (1.335) ± 0.186 long 1.25-1.75 (1.54) ± 0.188 wide. Ovary is oval shaped, located above cirrus sac on right side of the body and measuring 0.42-0.6 (0.514) ± 0.069 long 0.32-0.40 (0.374) ± 0.029 wide in width. Cirrus sac is short tube like structure found in right side of ovary. It is measuring 0.31-0.6 (0.473) ± 0.098 long 0.11-0.133 (0.113) ± 0.011 width. Seminal vesicle and ejaculatory duct are found near cirrus pouch. Genital atrium and Laurers canal present. Excretory vesicle small and excretory plexus extended in peripheral parenchymal pore. Eggs are numerous found in uterus. Vitellaria globular and distributed from lower edge of ventral sucker to posterior extremity. However, these are absent from midline of body.

Taxonomic summary

No. of specimens recovered: 46
No. of hosts found positive: 04
Site of infection: Esophagus and Gizzard
Etymology: Species name refers to village Awadh from where the host birds were collected.

DISCUSSION

Genus *Clinostomum* was created by Leidy, 1856 for accommodating *Distoma complanatum.* Stiles and Hassal, 1894 designated *C. gracile* as type species. The genus *Clinostomum* has remained center of attention for taxonomist for their different morphological characteristics within species. Many researchers have revised validity and status of many species. Ukoli, 1966 described 20 previously known species as synonymous of *C. complanatum*.

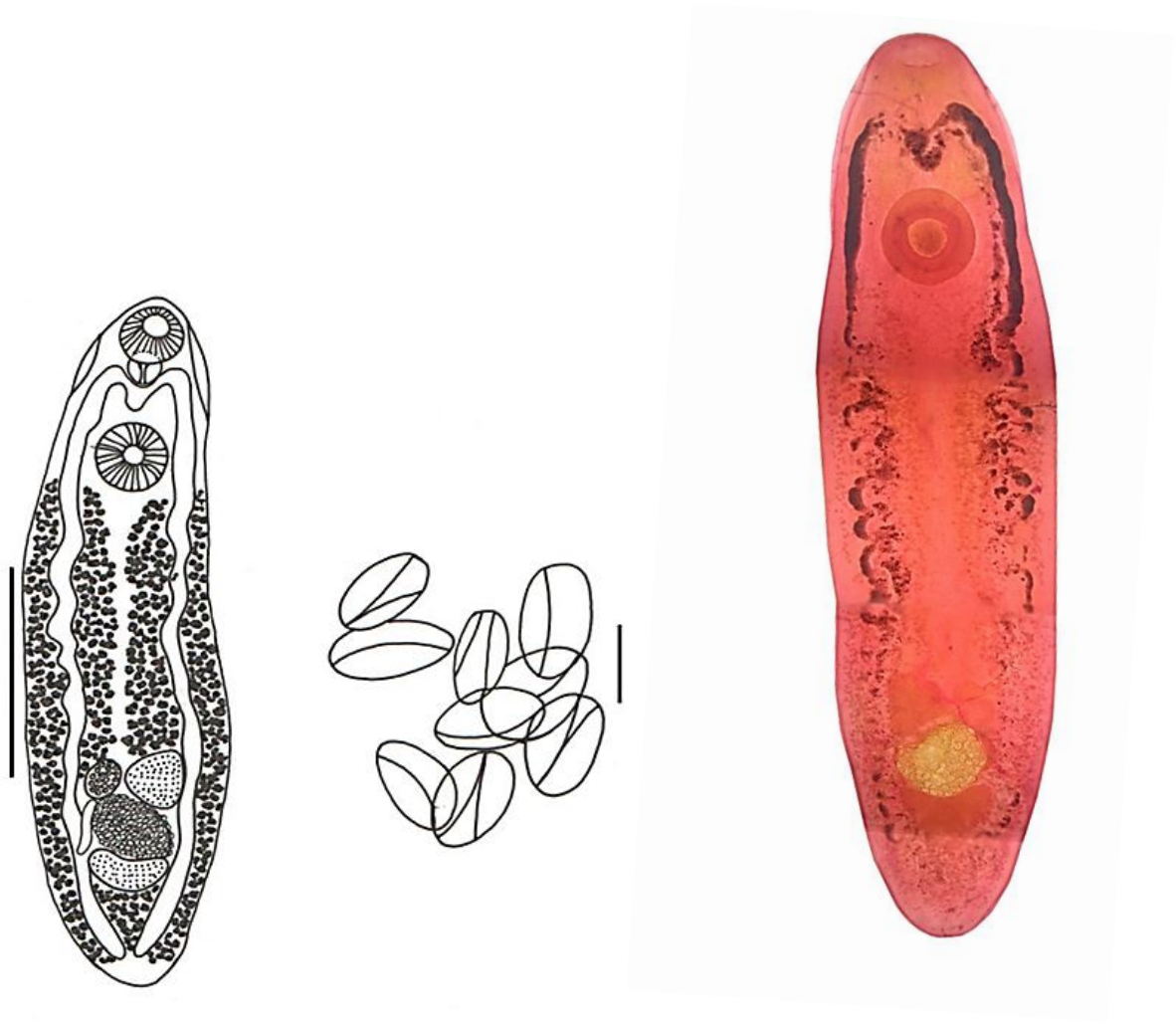

Fig 1. *Clinostomum awadhi* n. sp.; A. Entire worm; B. Eggs; C. Photograph of entire worm. **Scale bar:** A. 3 mm and B. 0.1 mm.

He recognized 13 valid species of this genus. Yamaguti, 1971 did not agree with Ukoli, 1966 completely. He described 26 valid species. Later on, Feizullaev and Mirzoeva, 1983 demoted and synonymized all *Clinostomum* species with *C. complanatum* except *C. sorbens* Braun, 1899; *C. heluans* Braun, 1899; *C. detruncatum* Braun, 1899; *C. ophicephali* Tubangui and Masilungan, 1944; *C. philippinense* Velasquez, 1960 and *C. phalacrocoracis* Dubois, 1931. Matthews and Cribb, 1998 revalidated *C. australiense* Johnston, 1917 and *C. hornum* Nicoll, 1914 during his study on *Clinostomum* in Australian fish-eating birds and described a new species *C. wilsoni*. Ongoing debate on status and validity of species within genus *Clinostomum* have been supplemented with molecular data by many researchers including Dizkowski et al. 2004, Gustinelli et al. 2010, Bonett et al. 2011, Caffara et al. 2011, Sereno-uribe et al. 2013, Pinto et al. 2015, Locke et al. 2015 and Acosta et al. 2016. However, morphological criteria still play fundamental role in classifying and describing species. This attempt to describe new species is purely based on morphological approach. Present form has typical features including size and position of uterus, location, arrangement and size of ovary, testes, cirrus sac, distribution of vitellaria and variation in posterior and anterior extremities. It is compared with close related species of genus *Clinostomum* and found differing with them in certain features.

C. complanatum differs from present species in having maximum width in gonadic region, narrow anterior end, small oral sucker, triangular median testes, oval and inter-testicular ovary, cirrus sac lies on left margin of anterior testis, tubular uterus. *C. dasi* vary from present species in having smaller body, wider posterior, maximum width at mid-body, small oral sucker, multilobed testes, anterior testes lie on right of median line, ovary diagonal to cirrus sac found in between anterior and posterior testes and small and oval cirrus sac. *C. cuteneum* also show clear marks of differences with present

Table-1. Comparison of various species of genus *Clinostomum* with *Clinostomum awadhi* n.sp.

Name of organ	*C. awadhi* n.sp.	*C. Complanatum*	*C. dasi*	*C. cutaneum*	*C. singhi*	*C. intermediale*	*C. phalacrocoracis*	*C. marginatum*	*C. detuncatum*	*C. attenatum*
Reference	Present study	Caffara et al. 2011	Al-Salim and Ali, 2010	Gustinelli et al. 2010	Bhutta and Khan, 1975	Lamont, 1920	Caffara et al. 2014	Caffara, 2011	Acosta et al. 2016	Jaiswal, 1957
Body	tongue shaped 8-10 (8.93) ± 0.694 long, 2.7-3.33 (3.003) ± 0.199, wide wider at equator	Stout, oval, elongated, wider in gonadic region (3.4-6.3 X 1.5-2.7)	tongue shaped, (4.4 X1.5) maximum width at mid –body	tongue shaped, (6.16 X 2.14), posterior end broad	oblong shaped, broadly rounded ends (2.727-3.636 X 1.151-1.363)	oblong shape, (7X I.5)	stout, slightly wider in gonadic region (9.5-15.2 X 1.8-3.9)	oval, elongated, wider at gonads (5.9-8.2 X 1.3-2.8)	long and robust (8.022-9.27 X 3.220-3.69)	(5 X 1.1)
Oral sucker	spherical, sub- terminal 0.65-0.86 (0.75) ± 0.071 long 0.73-0.933 (0.83) ± 0.073	small (0.19-0.570 X 0.32-0.850)	small, rounded, Sub-terminal	small (0.29 X 0.394)	sub-terminal, spherical (0.392 X 0.392)	well develop, sub-terminal	(0.41-0.533 X 0.478-0.732)	Small (0.171-0.394 X 0.252-0.5	Small (0.315-0.405 X 0.421-0.441)	(0.18 X 0.14)
Ventral Sucker	spherical and large 0.97-1.0 **(**0.981) ± 0.0184	large and spherical (0.70-0.90 X 0.62-0.90)	larger, spherical	large and round (0.855 X 0.878)	large (0.727 X 0.757)	well developed	(0.926-1.253 X 1.011-1.346)	(0.601-0.918 X 0.58-0.966)	Triangular opening (1.02-1.072 X 1.049-1.14)	(0.68 X 0.62)
Esophagus	short 0.533-0.632 (0.584) ± 0.034 long	short and bulbous	short bulbous	Short	Short	short	short and evident	Very short	Short	--
Ceca	bifurcated, extended from ventral sucker to posterior end	bifurcated, extended from ventral sucker to posterior end	bifurcated, extended from ventral sucker to posterior end	bifurcated, extended from ventral sucker to posterior end	bifurcated extended from ventral sucker to posterior end	bifurcated, extended from ventral sucker to posterior end	bifurcated extended from ventral sucker to posterior end	bifurcated run up to posterior end	robust, lateral and bifurcated up to posterior end	bifurcated run up to posterior end
Testes	irregularly lobed in hindbody. posterior larger than anterior but obscured by uterus. Anterior testis 0.85-0.933(0.887)± 0.027 long, 0.70-0.80 (0.747)± 0.0355 wide Posterior testis 0.35- 0.46 (0.4) ± 0.037 long, 0.95-1.20 (1.051) ± 0.092 wide	triangular anterior testis (0.550-0.756 X 0.360-0.600) posterior testis (0.60-0.94 X 0.30-0.510)	multilobed, in midbody, anterior testis on right side of median line	irregularly lobed Located in middle third of body	highly lobed, tandem, located in middle third post- acetabular region, Anterior testis (0.196 X 0.294) posterior testis larger (1.96-0.245 X 0.421-0.441)	testes are divided distinctly into three lobes situated in mid-line in posterior third of body	found middle and posterior third of body. Anterior testis (0.677-1.466 X 0.643-1.469) in middle third, Posterior testis, (0.606-1.182 X 0.695-1.469)	triangular found in middle and posterior third of the body. Anterior testis (0.290-0.743 X 0.569-1.141) Posterior testes (0.166-0.587 X 0.379-1.141)	slightly lobed in posterior third of the body. Anterior testis (0.359-0.655 X 0.859-1.266) Posterior testis (0.528-0.768 X 0.802-1.318)	triangular, anterior testis (0.39 X 0.35) Posterior testis (0.35 X 0.34)
Ovary	small, oval, right side of midline above cirrus sac 0.42-0.6 (0.514) ± 0.069 long 0.32-0.40 (0.374) ±0.029 wide	oval, intertesticular (0.22-0.310 X 0.14-0.301s	small, diagonal to cirrus sac in between testes	small, irregular in shape	irregular shaped, submedian lying just behind cirrus sac	ovoid shape lies between cirrus sac it and the posterior testis	---------	small, ovoid on right side of body (0.118-0.36 X 0.101-0.267)	small ovoid intertesticular (0.157-0.205 X 0.102-0.155)	oval (0.09 X0.10)
Cirrus sac	bean shaped, below ovary and right side of uterus (0.31-0.6 (0.473) ± 0.098 long 0.11-0.133 (0.113) ± 0.0111	right margin of anterior testis (0.356-0.40 X 0.10-0.20)	oval, small between anterior and posterior testes	large, rounded and deep clef formed two lobes	oval, located posteriorly to anterior testis	cirrus sac lies between testes and not anterior to them	bean-shaped (0. 389-0.717 X 0.143-0.292)	anterior to ovary (0.151-0.79 X 0.166-0.212	half-moon shape, near fright anterior margin of anterior testes (0.604-0.685 X 0.180-0.225)	---
Uterus	irregular in hindbody between anterior and posterior testes1.01-1.55 (1.335) ± 0.186 long 1.25-1.75 (1.54) ± 0.188	irregular between acetabulum to anterior testis	irregular between acetabulum and anterior testis	Y-shaped	narrow and elongated	lies between testes, reaches area between acetabulum and anterior testis	runs straight from ventral sucker to anterior testis	runs from left margin of anterior testis up to ventral sucker	uterine with lateral evaginations	---
Host	*Phalacrocorax niger*	*Ardea Herodias* and *Bubulcus ibis*	*Botaurus stellaris*	*Ardea cinerea*	*Ardeola grayi*	*Phalacrocorax vigua*	*Cichlids*	*Ardea Herodias* and *Bubulcus ibis*	*Synbranchus marmoratus*	*Rana tigrina*
Locality	Sindh, Pakistan	Quebec, Canada and Frida and Taxas	South Iraq	Kenya	Peshawar, Pakistan	Michigan, USA	Israel	Quebec, Canada, Florida and Taxas	Brazil	India

species in having smaller body, posterior end broad, small oral and ventral suckers, testes situated in middle third of body, ovary irregular shaped, cleft formed cirrus sac and Y shaped uterus. *C. singhi* differs from present species by having oblong shaped smaller body, broadly rounded ends, small oral and ventral suckers, slightly lobed testes, smaller anterior testes, large posterior testes, irregular shaped ovary lying behind cirrus sac, narrow and elongated uterus. *C. intermadiale* is remarkable different from present species in having oblong shaped smaller body, trilobed testes, ovary situated between cirrus sac and posterior testes, cirrus sac lies between testes. *C. phalacrocoracis* differs from present species by having stout shaped larger body with maximum width at gonadic region, small oval sucker, slightly wider ventral sucker, wider anterior testes, long posterior testes and larger cirrus. *C. attentum* reported from *Rana tigrina* differs from present species in having smaller body, smaller oral and ventral suckers, triangular shaped smaller testes and ovary. *C. demiegrettae* differs from present species in having slightly attenuated anterior end, broader posterior end, small oral sucker, cylindrical oesophagus, multilobed testes located in middle third of post-acetabular portion slightly different in size and smaller ovary. *C. hyderabadensis* differs from present species by having oval shaped smaller body, small oral and ventral suckers, cylindrical esophagus, triangular shaped testes, small anterior testis and longer posterior testis, small pear shaped ovary and rounded follicular vitellaria.

Clinostomum marginatum is close to present species in size of body (5.9-8.2 X 1.3-2.8) but differ in having small oral sucker (0.17-0.394 X 0.601-0.96), testes triangular found in mid-body, anterior testis (0.29-0.743 X 0.569-1.141), posterior testis (0.166-0.587 X 0.374-1.141), small ovary located in right side of body, cirrus sac is anterior to ovary and uterus run up to post-acetabular region.

C. detruncatum differ from present species in having robust body, small oral sucker (0.315-0.405 X 0.421-0.441), triangular opening of ventral sucker, shape and size of testes, anterior testis (0.359-0.655 X 0.859-1.26), posterior testis (0.528-0.768 X 0.802-1.318), inter-testicular and small ovary (0.157-0.205 X 0.102-0.155), half-moon shaped cirrus sac and lateral evagination of uterus.

CONCLUSION

Present study recorded a new Clinostome species. It was compared with close related species *C. Complanatum*, *C. dasi*, *C. cutaneum*, *C. singhi*, *C. intermediale*, *C. phalacrocoracis*, *C. marginatum*, *C. detuncatum* and *C. attenatum* (Table no. 1). Therefore, *Clinostomum awadhi* differs with congeners in size of body, maximum width, size and shape of posterior and anterior extremities, shape and location of uterus, cirrus sac, location of ovary and distribution of vitellaria. Therefore, present fluke is identified as a new species and named *Clinostomum awadhi*. Species name awadhi refers to the name of locality from where the host birds were collected.

REFERENCES

Abro MM, Dharejo AM, Khan MM and Birmani NA. 2016a. First record of *Clinostomum Complanatum* (Trematodes: Clinostomatidae) in Pakistan from *Phalacrocorax niger* (Aves: Phalacrocoracidae). Biological Forum–An International Journal. 8(1): 479-483.

Abro MM, Dharejo AM, Khan MM and Birmani NA. 2016b. A new species of genus *Paryphostomum* Dietz, 1909 (Trematoda: Echinostomatidae) in *Phalacrocorax niger* of Pakistan. Journal of Entomology and Zoology Studies. 4(3): 246-249. http://www.entomoljournal.com/archive/2016/vol4issue3/PartD/4-3-75.pdf

Abro MM, Dharejo AM, Khan MM and Birmani NA. 2016c. A New record of Genus *Macrobilharzia* Travassos, 1922 (Trematoda: Schistosomatoidea) in *Phalacrocorax niger* from Sindh, Pakistan. Journal of Entomology and Zoology Studies. 4(4): 654-656.

Abro MM, Dharejo AM, Khan MM and Birmani NA. 2016d. *Euclinostomum heterostomum* (Rud., 1809) Travassos, 1928 (Trematodes: Clinostomidae: Euclinostominae): a new record in avian host Little cormorant (Aves: Phalacrocoracidae) of Pakistan.Journal of Advances in Biology and Biotechnology. 8(1): 1-5. Article No.JABB.27782.

Abro MM, Dharejo AM, Khan MM and Birmani NA. 2016e. New host and locality record of *Paryphostomum radiatum* (Dujardin, 1845) (trematodes: Echinostomatidae) from Pakistan. Journal of Biology and Nature. 6(2): 104-108.

Acosta AA, Caffara M, Fioravanti ML, Cristina Zago RUA, Franceschini L and Silva R J. 2016. Morphological and Molecular Characterization of *Clinostomum detruncatum* (Trematoda: Clinostomidae) Metacercariae Infecting *Synbranchus marmoratus*. Journal of Parasitology. 102(1): 151-156.

Akram M. 1996. *Contracaecum bubakii* new species (Nematoda: Anisakidae) from the Cormorant in Pakistan. Pakistan Journal of Zoology. 28: 131-132.

AL-Salim NK and Ali AH. 2010. First record of three species of trematodes of the genus *Clinostomum* Leidy 1856 (Digenea: Clinostomidae) parasitic in Piscivorous

birds from East AL-Hammar Marsh, South of Iraq. Marsh Bulletin. 5(1): 27-42.

Bhutta MS and Khan D. 1975. Digenetic trematodes of vertebrates from Pakistan. Bulletin Department of Zoology University of Punjab, (N.S.) Article 8: 1-175.

Bonett RM, Steffen MA, Trujano-Alvarez AL, Martin SD, Bursey CR and McAllister CT. 2011. Distribution, abundance, and genetic diversity of *Clinostomum* spp. metacercariae (Trematoda: Digenea) in a modified Ozark stream system. Journal of Parasitology. 97:177–184

Bullard SA and Overstreet RM. 2008. Digeneans as enemies of fishes. *In Fish diseases*. Eiras, J., H. Segner, T. Wahil, and B. G. Kapoor. (eds). Science Publishers. Enfield, New Hampshire. 817-976.

Caffara MG, Bruni C, Paoletti A, Gustinelli and Fioravanti ML. 2014. Metacercariae of *Clinostomum complanatum* (Trematoda: Digenea) in European newts *Triturus carnifex* and *Lissotriton vulgaris* (Caudata: Salamandridae). Journal of Helminthology. 88: 278-285.

Caffara MSA, Locke A, Gustinelli DJ, Marcogliese and Fioravanti M L. 2011. Morphological and molecular differentiation of *Clinostomum complanatum* and *Clinostomum marginatum* (Digenea: Clinostomidae) metacercariae and adults. Journal of Parasitology. 97: 884-891.

Chung D, Kong HH and Moon CH. 1995. Demonstration of the second intermediate hosts of *Clinostomum Complanatum* in Korea. Korean Journal of Parasitology. 33(4): 305-312.

Dharejo AM, Birmani NA and Khan MM. 2010. First record of the genus *Nigerina* Baugh, 1958 (Trematoda: Opisthorchidae) from Pakistan in avian host little cormorant, *Phalacrocorax niger*. Proceedings of Parasitology. 50: 147-151.

Dzikowski RMG, Levy MF, PooreJR, Flowers and Paperna. 2004. *Clinostomum complanatum* and *Clinostomum marginatum* (Rudolphi, 1819) (Digenea: Clinostomidae) are separate species based on differences in ribosomal DNA. Journal of Parasitology. 90: 413-414.

Eiras JC. 1994. A importânciaeconômica dos parasitas de peixes. Higi Aliment. 8(31): 11-13.

Feizullae NA and Mirzoeva SS. 1983. Revision of the Superfamily Clinostomoidea and analysis of its system. Parazitol. 17: 3-11 (in Russian).

Gibson DI, Jones A and Bray RA. 2002. Keys to the trematoda Vol.1. CABI Publishing and Natural History Museum, London, UK.

Gustinelli A, Caffara M, Florio D, Elick O, Otachi EO, Euty M, Wathuta EM and Fioravanti ML. 2010. First description of the adult stage of *Clinostomum cutaneum* Paperna, 1964 (Digenea: Clinostomidae) from grey herons Ardeacinerea L. and a redescription of the metacercaria from the Nile tilapia *Oreochromis niloticus niloticus* (L.) in Kenya. Systematic Parasitology. 76: 39-51. DOI10.1007/ s11230-010-9231-5.

Jaiswal PS. 1957. Studies on the trematodes of fishes and birds found in Hyderabad state. Osmania university, Hyderabad Deccan (India).

Lamont ME. 1920. A new species of *Clinostomum*. Occasional paper of the Museum Zoology, University of Michigam. 83.

Lo CF, Wang CH, Huber F and Kou GH. 1982. The study of *Clinostomum complanatum*. CAPA Fisheries Series No. 8. Fish Diseases Research. 4: 26-56.

Locke SA, Caffara M, Marcogliese DJ and Fioravanti ML. 2015. A large-scale molecular survey of *Clinostomum* (Digenea, Clinostomidae). Zoologica Scripta. 44: 203-217.

Matthews D and Cribb TH. 1998. Digenetic trematodes of the genus *Clinostomum* Leidy, 1856 (Digenea: Clinostomidae) from birds of Queensland, Australia, including *C. wilsoni* n. sp. from *Egretta intermedia*. Systematic Parasitology. 39: 199-208.

Olsen WO. 1974. Animal parasites: Their life cycles and ecology. Baltimore: University Park Press. 562.

Paperna I. 1996. Parasites Infections and disease of fish in Africa. An update, CIFA Tech. Report. 31: 220.

Pinto HA, Caffara M, Fioravanti ML and Melo AL.2015. Experimental and molecular study of cercariae of *Clinostomum* sp. (Trematoda: Clinostomidae) from *Biomphalaria* spp. (Mollusca: Planorbidae) in Brazil. Journal of Parasitology. 101(1):108-13. doi:10.1645 /14-497.

Price EWA.1938. Redescription of *Clinostomum intermedialis* Lamont (Trematoda: Clinostomidae) with a key to the species of the genus. Proceedings of Helminthological Society of Washington. 5: 11-13.

Rais M, Khan ZM, Abbas D, Akber G, Nawaz R and Saeed-ul-Islam. 2011. A quantitative study on wildlife of Chotiari reservoir Sanghar, Sindh, Pakistan. Pakistan Journal of Zoology. 42(2): 237-247.

Roberts TJ. 1991.The Birds of Pakistan. Vol. I. Non-Passeriformes. Oxford University Press. Karachi. 598

Sarker NJ and Naher H. 2002. Experimental studies on food habits of the little cormorant, *Phalacrocorax niger* (Vieillot).

Bangladesh Journal of Zoology. 302:173-182.

Sereno-Uribe AL, Pinacho-Pinacho CD, García-Varela M and León GP. 2013. Using mitochondrial and ribosomal DNA sequences to test the taxonomic validity of*Clinostomum Complanatum* Rudolphi, 1814 in fish-eating birds and freshwater fishes in Mexico, with the description of a new species. Parasitology Research. 112: 2855-2870.

Sutili FJ, Gressler TL and Pelegrini LV. 2014. *Clinostomum complanatum* (Trematoda, Digenea): a parasite of birds and fishes with zoonotic potential in southern Brazil. A RevieuRevistaBrasileira de Higiene e Sanidade Animal 08 (1): 99-114. http://dx.doi.org/10.5935/19812965. 20140007.

Szalai AJ and Dick TA. 1988. Helminths of stocked rainbow trout (*Salmo gairdneri)* with special references to *Clinostomum complanatum*. Journal of Wildlife Diseases; 24(3): 456-460.

Tiewchaloern S, Udomkijdecha S, Suvouttho S, Chunchamsri K and Waikagul J. 1999. *Clinostomum* trematode from human eye. Southeast Asian Journal of Tropical Medicine and Public Health. 30: 382-384.

Thatcher VE. 198. Patologia de peixes da Amazônia brasileira, 1. *Aspectos gerais.* Acta Amazon. 11: 125-140.

Ukoli FMA. 1966. On *Clinostomum tilapiae* n.sp., and *C. phalacrocoracis* Dubois, 1931 from Ghana, and a discussion of the systematics of the genus *Clinostomum* Leidy, 1856. Journal of Helminthology. 40: 187-214.

Yamaguti S. 1971. Synopsis of digenetic trematodes of vertebrates Vol. I Keigaku Publishing Co. Tokyo, Japan.; 1575.

Yoshimura K, Ishigooka S, Satoah I and Kamegai S. 1991. *Clinostomum complanatum* from the pharynx of a woman in Akita, Japan. Japan Journal of Parasitology. 40: 99-101.

Zeenath C. 2009. Behavior and adaptation of little cormorant *Phalacrocorax niger* and Darter *Anhinga melanogaster*. Ph. D. Thesis. Post-graduate and Research Department of Zoology St. Joseph's College, Devagiri, Calicut. Kerala India. 202.