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

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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, Phalacrocorax 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, Nycanass, Larus, Batorides, Phalacrocorax, Babulcus, Botaurus, Plegadis, Gallinula, Podiceps, Iborychus, 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 niger* 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, Sarker 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 interceliac 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.*
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

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.
<table>
<thead>
<tr>
<th>Name of organ</th>
<th>C. awadhi n. sp.</th>
<th>C. Complanatum</th>
<th>C. dasi</th>
<th>C. cutaneum</th>
<th>C. singhi</th>
<th>C. intermedius</th>
<th>C. phalacrocoracis</th>
<th>C. marginatus</th>
<th>C. detunctam</th>
<th>C. attenuatum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body</td>
<td>Stout, oval, elongated, wider in gonadic region (3.4-6.3 X 1.5-2.7)</td>
<td>tongue shaped, (4.4 X 1.5) maximum width at mid-body</td>
<td>tongue shaped, (6.16 X 2.14), posterior end broad</td>
<td>oblong shaped, broadly rounded ends (2.727-3.636 X 1.151-1.363)</td>
<td>Stout, slightly wider in gonadic region (9.5-15.2 X 1.8-3.9)</td>
<td>oval, elongated, wider at gonads (5.9-8.2 X 1.3-2.8)</td>
<td>long and robust (8.022-9.27 X 3.220-3.69)</td>
<td>oval, elongated, wider at gonads (5.9-8.2 X 1.3-2.8)</td>
<td>Small (0.171-0.394 X 0.252-0.5)</td>
<td>Small (0.315-0.405 X 0.421-0.441)</td>
</tr>
<tr>
<td>Oral sucker</td>
<td>spherical, sub-terminal 0.65-0.86 (0.75) ± 0.071 long, 0.73-0.93 (0.83) ± 0.073</td>
<td>small (0.19-0.570 X 0.32-0.850)</td>
<td>small, rounded</td>
<td>sub-terminal, spherical (0.392 X 0.392)</td>
<td>(0.41-0.533 X 0.478-0.732)</td>
<td>well develop, sub-terminal</td>
<td>(0.601-0.918 X 0.58-0.966)</td>
<td>Triangular opening (1.02-1.072 X 1.049-1.14)</td>
<td>(0.18 X 0.14)</td>
<td>(0.68 X 0.62)</td>
</tr>
<tr>
<td>Ventral sucker</td>
<td>spherical and large 0.97-1.0 (0.981) ± 0.0184</td>
<td>large and spherical (0.70-0.90 X 0.62-0.90)</td>
<td>larger, spherical</td>
<td>large and round (0.855 X 0.878)</td>
<td>(0.727 X 0.757)</td>
<td>well developed</td>
<td>(0.926-1.253 X 1.011-1.136)</td>
<td>---</td>
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</tr>
<tr>
<td>Esophagus</td>
<td>short 0.533-0.632 (0.584) ± 0.034 long</td>
<td>short and bulbous</td>
<td>short bulbous</td>
<td>short</td>
<td>short</td>
<td>short</td>
<td>short and evident</td>
<td>Very short</td>
<td>Short</td>
<td>---</td>
</tr>
<tr>
<td>Ceca</td>
<td>bifurcated, extended from ventral sucker to posterior end</td>
<td>bifurcated, extended from ventral sucker to posterior end</td>
<td>bifurcated, extended from ventral sucker to posterior end</td>
<td>bifurcated, extended from ventral sucker to posterior end</td>
<td>bifurcated, extended from ventral sucker to posterior end</td>
<td>bifurcated, extended from ventral sucker to posterior end</td>
<td>bifurcated run up to anterior end</td>
<td>robust, lateral and bifurcated up to posterior end</td>
<td>bifurcated run up to posterior end</td>
<td>---</td>
</tr>
<tr>
<td>Testes</td>
<td>irregularly lobed in hindbody, posterior larger than anterior but obscured by uterus. Anterior testis 0.85-0.933(3.887a) ± 0.027 long, 0.70-0.80 (0.747a) ± 0.0355 wide</td>
<td>triangular anterior testis (0.550-0.756 X 0.360-0.600) posterior testis (0.60-0.94 X 0.30-0.510)</td>
<td>multilobed, in midbody, anterior testis on right side of median line</td>
<td>irregularly lobed Located in middle third of body</td>
<td>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)</td>
<td>tests are divided distinctly into three lobes situated in mid-line in posterior third of body</td>
<td>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)</td>
<td>triangular found in middle and posterior third of the body. Anterior testis (0.390-0.655 X 0.859-1.266) Posterior testis (0.526-0.768 X 0.802-1.318)</td>
<td>slightly lobed in posterior third of the body. Anterior testis (0.359-0.655 X 0.859-1.266) Posterior testis (0.526-0.768 X 0.802-1.318)</td>
<td>triangular, anterior testis (0.39 X 0.35) Posterior testis (0.35 X 0.34)</td>
</tr>
<tr>
<td>Ovary</td>
<td>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)</td>
<td>oval, intertesticular (0.22-0.310 X 0.14-0.301a)</td>
<td>small, diagonal to cirrus sac in between testes</td>
<td>small, irregular in shape</td>
<td>irregular shaped, submedian lying just behind cirrus sac</td>
<td>ovoid shape lies between cirrus sac it and the posterior testis</td>
<td>---</td>
<td>small, ovoid on right side of body (0.118-0.36 X 0.101-0.267)</td>
<td>small ovoid intertesticular (0.157-0.205 X 0.102-0.155)</td>
<td>oval (0.09 X 0.10)</td>
</tr>
<tr>
<td>Cirrus sac</td>
<td>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</td>
<td>right margin of anterior testis (0.356-0.4 X 0.10-0.20)</td>
<td>oval, small between anterior and posterior testes</td>
<td>large, rounded and deep cleft formed two lobes</td>
<td>ovular, located posteriorly to anterior testis</td>
<td>cirrus sac lies between tests and not anterior to them</td>
<td>bean-shaped (0.389-0.717 X 0.143-0.292)</td>
<td>anterior to ovary (0.151-0.79 X 0.166-0.212)</td>
<td>half-moon shape, near frakt anterior margin of anterior testis (0.604-0.685 X 0.180-0.225)</td>
<td>---</td>
</tr>
<tr>
<td>Uterus</td>
<td>irregular in hindbody between anterior and posterior testes 0.1-1.55 (1.335) ± 0.186 long 1.25-1.75 (1.54) ± 0.188</td>
<td>irregular between acetabulum and anterior testis</td>
<td>irregular between acetabulum and anterior testis</td>
<td>V-shaped</td>
<td>narrow and elongated</td>
<td>lies between testes, reaches area between acetabulum and anterior testis</td>
<td>runs straight from ventral sucker to anterior testis</td>
<td>runs from left margin of anterior testis up to ventral sucker</td>
<td>uterine with lateral evaginations</td>
<td>---</td>
</tr>
<tr>
<td>Host</td>
<td>Phalacrocorax nigerr</td>
<td>Ardea Herodias and Bubalubs ibis</td>
<td>Botaurus stellaris</td>
<td>Ardea cinerea</td>
<td>Ardeola grayi</td>
<td>Phalacrocorax vigua</td>
<td>Cichlids</td>
<td>Ardea Herodias and Bubalubs ibis</td>
<td>Synbranchus marmoratus</td>
<td>Rana tigrina</td>
</tr>
<tr>
<td>Locality</td>
<td>Sindh, Pakistan</td>
<td>Quebec, Canada and Frida and Taxas</td>
<td>South Iraq</td>
<td>Kenya</td>
<td>Peshawar, Pakistan</td>
<td>Michigan, USA</td>
<td>Israel</td>
<td>Quebec, Canada, Florida and Taxas</td>
<td>Brazil</td>
<td>India</td>
</tr>
</tbody>
</table>
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. intermediale* 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. demieggrettae* 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. hyderabadiensis* 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.

*Clinostomum 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. detruncatum* and *C. attentatum* (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.

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