



## **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**

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### **Abstract**

Three species of trematodes, genus *Clinostomum* Leidy, 1856 were described from three different species of piscivorous birds (*viz.* *C. complanatum* Leidy, 1856 from grey heron *Ardea cinerea* and from small white Bittern *Ardeola ralloides*, *C. dasi* Bhalerao, 1942 from Bittern *Botaurus stellaris* and *Clinostomum* sp. from each of *A. cinerea* and *A. ralloides*). Birds were captured from East Al-Hammar marsh, south of Iraq during December-February 2004 and December 2005. The genus *Clinostomum* and its three species were recorded and described as adult for the first time in Iraq.

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### **1- Introduction**

Members of *Clinostomum* Leidy, 1856, parasite of buccal cavity of aquatic birds, used aquatic snail and fishes (rarely frogs and toads) as first and the second intermediate hosts respectively (Lo *et al.*, 1982).

Metacercariae of *Clinostomum* spp. Have been reported from freshwater fishes of

Iraq, but there is no report on adult stage from water birds, only two specimens of immature *C. phalacrocoracis* Dubios, 1931 was isolated from proventriculous (unusual site for *Clinostomum* to probability the bird as final host) of pygmy cormorant from Al-Sweeb Marsh North east of Basrah province by Al-Mayah (1994). Because of shortage of knowledge about final host in

Iraq and such worms infect freshwater and pond fishes (Ali, 2001) this work was done.

## 2- Materials and Methods

Twelve Grey heron *Ardea cinerea*, 7 Bittern *Botaurus stellaris*, 3 small white heron *Ardeola ralloides* were collected from two stations, West Al-Hammar Marsh near Suq Al-Shuyukh city (30° 51' N and 40° 46' E), south of Thi Qar province and East Al-Hammar Marsh north of Basrah province (30° 40' N and 47° 33' E) during December-February 2004 and December 2005. The former bird was collected from East Hammar, while the rest were collected from west Al-Hammar marsh. The birds were shot, each individual preserved with ice bags. Some birds transferred to the laboratory alive.

Birds were classified according to Allous (1960) and Porter *et al.*(1996). Buccal cavity of each bird was detected for

parasite, the parasites were synthesized by drops of 70 % ethanol, fixed in hot 4% formaldehyde , preserved in 70% ethanol, stained with acid carmine and mounted in canada balsam. All drawings were established by aid of a camera lucida fixed on compound microscope type Yaseen at 40-1600 magnification. All measurements are in micrometer unless otherwise stated. Specimens were deposited in the laboratory of parasite of fishes, department of fisheries and marine resources, college of Agriculture.

## 3- Results

Three species of adult digenetic trematode of the genus *Clinostomum* were recorded and described from piscivorous birds, trematodes species and site of infection in the final hosts clarified in table 1.

**Table (1): Trematodes species, their hosts and site of infection.**

parasite	Site of infection	Bird
<i>C. complanatum</i>	Buccal cavity	<i>A. cinerea</i>
	Pharynx, tongue	<i>A. ralloides</i>
<i>C. dasi</i>	Buccal cavity	<i>B. stellaris</i>
<i>Clinostomum</i> sp.	tongue	<i>A. cinerea</i> and <i>A. ralloides</i>

#### 4- Descriptions

##### 1- *Clinostomum complanatum* Rud., 1814

##### Description based on 19 specimens (Fig. 1)

Tongue-like body (Fig.1A), concave ventrally 3935-10979(7472) in length. Posterior end wider than anterior end, maximum width 1152-3156(2236) at testes level, the ratio of the length to maximum width 2.2-5.2: 1 (3.4:1). The body has clear constriction at acetabulum level, oral collar well developed 587-978(770) in width, oral sucker small, oval subterminal, 270-474 (356) in length and 261-587(408) in width. Percentage of the oral sucker width to body width 12.5-29.7 (18.9) %. Fore body 695-1674(1129). Acetabulum situated in the last half of the first third part of body 360-1420(793) in length and 463-1394(1065) in width, ratio of width of acetabulum to oral sucker width 1.63-2.6:1(2:1), the percentage of acetabulum width to body width 30.1-54.7(40) %. The distance between the suckers 342-1261(774).

Esophagus bulb present, esophagus short bifurcated just anterior to acetabulum. Caeca extended to posterior of the body where it is connected to v- shaped excretory bladder. Each caeca has many diverticula on external and internal sides, in live specimens the caeca appear brownish or reddish in colour.

Testes (fig. 1A,B) triangle shape

multilobe, anterior testis turn to left from median longitudinal axis (asymmetrical) and located in posterior second third of body 326-1565(760) in length and 495-1210(893) in width, ratio of testis width to its length is 1-2.1(1.3). Posterior testis was symmetrical about the median longitudinal axis 360-717(558) in length and 558-1326(1006) in width, ratio of testis width to its length is 1.3-3:1(1.8:1), the distance between testes 135-869(457). Cirrus sac oval (fig. 1B), small 198-721 (563) in length and 180-360(272) in width, between anterior testis and caecum and just above ovary. Genital pore in anterior distal part of cirrus sac opposed to posterior side of anterior testis. Percentage ratio of cirrus length to body length 5.03-13.3(7.6) %. Ovary oval in shape, behind cirrus sac between testes 135-618(382) in length and 162-504(302) in width. Ratio of ovary width to its length is 0.43-1.5:1(0.81:1), ratio of cirrus sac length to ovary length 1.15-1.93:1(1.5:1).

Vitellaria global or uniform 51-73×51-78(60×75) in measurement, numerous with two lateral fields in distribution except thin circumoral belt absence from it. Vitellaria occupy the space behind acetabulum until posterior end of body. Vitellarial canal and vitellarial receptacle are present between testes, uterus canal open in uterus sac, the latter occupy the area between acetabulum

and anterior testis longitudinally and between caeca transversally, 756-3152(1666) in length and 198-935(516) in

width. Matured eggs (Fig.1C) 112-135(122)×65-81(72) in measurement in uterus and immature eggs between testes.

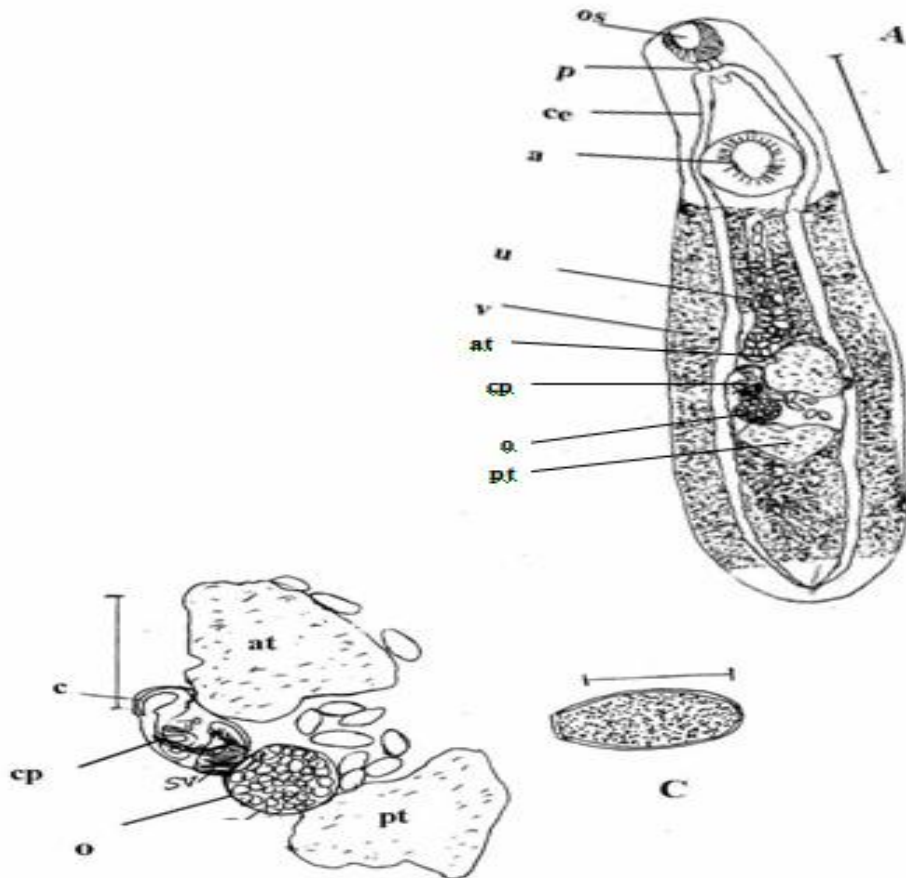


Fig.(1): *Clinostomum complanatum* ventral view, A: whole body, B: reproductive system, C: egg from uterus: acetabulum (a), anterior testis (at), cirrus (c), caecum (ce), cirrus pouch (cp), ovary (o), oral sucker (os), pharynx (p), posterior testis (pt), seminal vesicle (sv), uterus (u), vitellaria (v), Scale bar: A=1mm, B=450µm,

## 2- *Clinostomum dasi* Bhalerao, 1942

### Description based on one specimen (Fig. 2)

Tongue-like body (Fig. 2A), concave ventrally 4415 in length, posterior end wider than anterior end, maximum width 1509 in midbody (mid of anterior testis), ratio of the length to maximum width 2.9:1, with fine constriction at acetabulum level. Oral collar well developed 396 in width, oral sucker small, rounded subterminal, 226 in the diameter, percentage of oral sucker width to body width 14.97 %. Fore body 651. Acetabulum larger than oral sucker 471 in length and 547 in width in mid of the first third of body, ratio of width of acetabulum to oral sucker width 2.4:1, the percentage of acetabulum width to body width 36.6 %. The distance between the suckers 386.

Esophagus bulb present, esophagus short bifurcated just anterior to acetabulum, caeca extended to posterior of the body where it is connected to v- shaped excretory bladder, each caeca has heavily diverticula on both sides.

Testes (fig.1A,B) multilobed, anterior testis in middle of second third of body 471 in length and 792 in width, ratio of testis width to its length is 1.68:1. Posterior testis between second and posterior third of body, but most of it in the second third 566 in length and 792 in width, ratio of testis

width to its length is 1.5:1, the distance between testes 339. Cirrus sac (fig.2B) oval, small 283 in length and 169 in width, between anterior testis and caecum and diagonal with ovary, genital pore in anterior distal part of cirrus sac close to anterior testis. Percentage ratio of cirrus length to body length 6.4 %, ovary uniform in shape, diagonal with cirrus sac between testes 264 in length and 151 in width. Ratio of ovary width to its length is 1: 0.57, ratio of cirrus sac length to ovary length 1: 1.07.

Vitellaria global, numerous with two lateral fields in distribution except two thin circumoral belts are absent from it, Vitellaria occupy the space between posterior third of acetabulum level until relatively posterior end of body, vitellarial canal and vitellarial receptacle are present between testes, uterus canal open in uterus sac, the latter occupy the space between acetabulum and anterior testis longitudinally and between caeca transversally, Matured eggs (Fig. 2C) 117-126(122) in uterus and immatured eggs between testes.

## 3- *Clinostomum* sp.

### Description based on three specimen ( 2 complete and one fragment)

Elongated oval body (fig. 3A) 8435-9326(8880) in length, maximum width 1956-2500(2228) at testes region, hind

extremity boarder than anterior one, ratio of length of body to maximum width 3.7-4.3:1(4:1). Constriction not found in acetabulum region, , oral collar well developed 869-1000(949) in width, oral sucker small and oval, subterminal 304-456(358) in length and 391-478(436) in width, percentage ratio of oral sucker to body width 17.6-24.4(21)%. Fore body 1326-1717(1586). Acetabulum larger than oral sucker 810-956(894) in length and 783-846(814) in width in the posterior part of first third of body, many glandular cells in preacetabular region, ratio of width of acetabulum to width of oral sucker 1.91-2:1(1.95:1). Percentage ratio of acetabulum width to body width 33.8-38(36) %, the distance between suckers 879-1326(1144).

Esophagus present, short bifurcated just anterior of acetabulum, caeca extended to posterior of body where connected to v shape excretory vesicle, diverticula in both sides of caeca restricted to post testes region (fig. 3A).

Testes (fig. 3A,B) multilobed wider than

long, both in the same median of longitudinal axis, anterior testis in posterior part of second third of body 513-765(672) in length and 630-935(833) in width, ratio of anterior testis width to its length is 1.2-1.26:1(1.23:1). Posterior testis in the anterior part of posterior third of body 369-478(423) in length and 666-1413(1040) in width, ratio of posterior testis width to its length is 1.8-2.9:1 (2.3:1), the distance between testes 495-848(671).

Cirrus sac oval (fig. 3B) 495-887(729) in length and 288-333(315) in width and have constriction attached to caeca in side and diagonal with anterior testis above and diagonal bellow with ovary, posterior of cirrus sac elapsed with ovary. Percentage ratio of cirrus sac length to body length 5.8-9.4(7.6) %, genital pore in the distal part of cirrus sac close to posterior corner of anterior testis. Ovary crescent like 360-540(459) in length and 72-180(132) in width between testes, ratio of ovary width to its length 1.37-1.68:1(1.56:1).

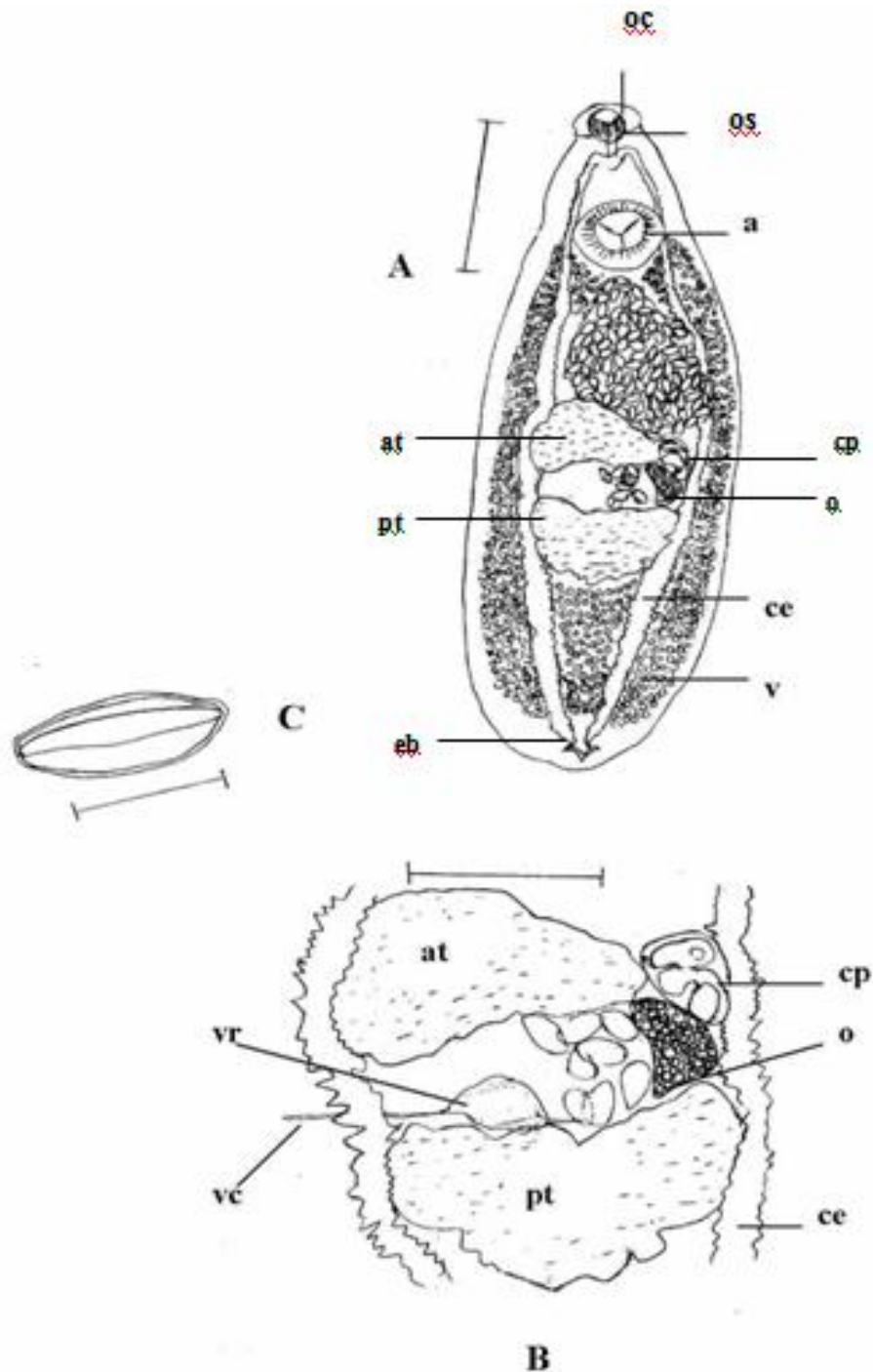


Fig.(2): *Clinostomum dasi* dorsal view, **A**: Whole body, **B**: reproductive system, **C**: egg from uterus. acetabulum (a), anterior testis (at), caecum (ce), cirrus pouch (cp), excretory bladder (eb), ovary (o), oral collar (oc), oral sucker (os), posterior testis (pt), vitellaria (v), vesicle canal (vc), vesicle receptacle (vr). Scale bar: **A**=1mm, **B**=450µm, **C**=90µm.

Vitellaria global and numerous in two lateral fields except two thin circumoral belts were absent from it. vitellaria occupy the space between posterior of acetabulum level until relatively posterior end of body and in median area between caeca. Vitellarial receptacle present between testes, uterus sac short and narrow 1522-2913(2203) in length and 217-478(340) in width occupy 79 % the space between actabulum and anterior testis contained a few eggs (matured)(fig. 3C) 112×56-79 in measurement.

#### 4-Discussion

##### 1- *Clinostomum complanatum* Rud., 1814

*Clinostomum* Leidy, 1856 established beside *Clinostomoides* Dollfus, 1950 and *Clinostomatopsis* Dollfus, 1932 in subfamily Clinostominae Luhe, 1901 because its stout body, linguiform, about 5-30 mm long and adults in buccal cavity and esophagus of birds (Kanev *et al.*, 2002).

This parasite caused yellow grub disease for fishes and causes high mortality in fry fishes in Taiwan (Lo *et al.*, 1981, 1985), or cultured rainbow trout (Szali and Dick, 1988) or fingerling of Cichlid on East Mediterranean and African countries

(Paperna, 1996). Also it caused "Halzoun disease of men after consuming raw infected fish (Yoshimura *et al.*, 1991 and Chung *et al.*, 1995), and it may infect the eye and caused inflammation (Tiewchaloern *et al.*, 1999).

Ukoli (1966) described adult and larval stages of *C. tilapiae* Ukoli, 1966 from buccal cavity for cattle egret *Bubulcus ibis* after experimentally fed it with infected Tilapia fish in Nigeria. He discuss briefly systematic position of genera in Clinostomatidae and focused on *Clinostomum* species and he revised the genus and created key to 13 valid species after rejected a larval species described from fishes, he placed 20 species as synonyms of *C. complanatum*. Feizuleav and Mirzaeva (1983, 1986) reviewed all previous studies on *Clinostomum* and examined hundreds of specimens and concluded that only a single species in the genus (*C. complanatum*) and considered all diagnostic characters for distinguished the species were unreliable. Ten years later species identifiable as belonging to *Clinostomum* seem to be referred routinely to *C. complanatum* (except rare cases) by most authors.



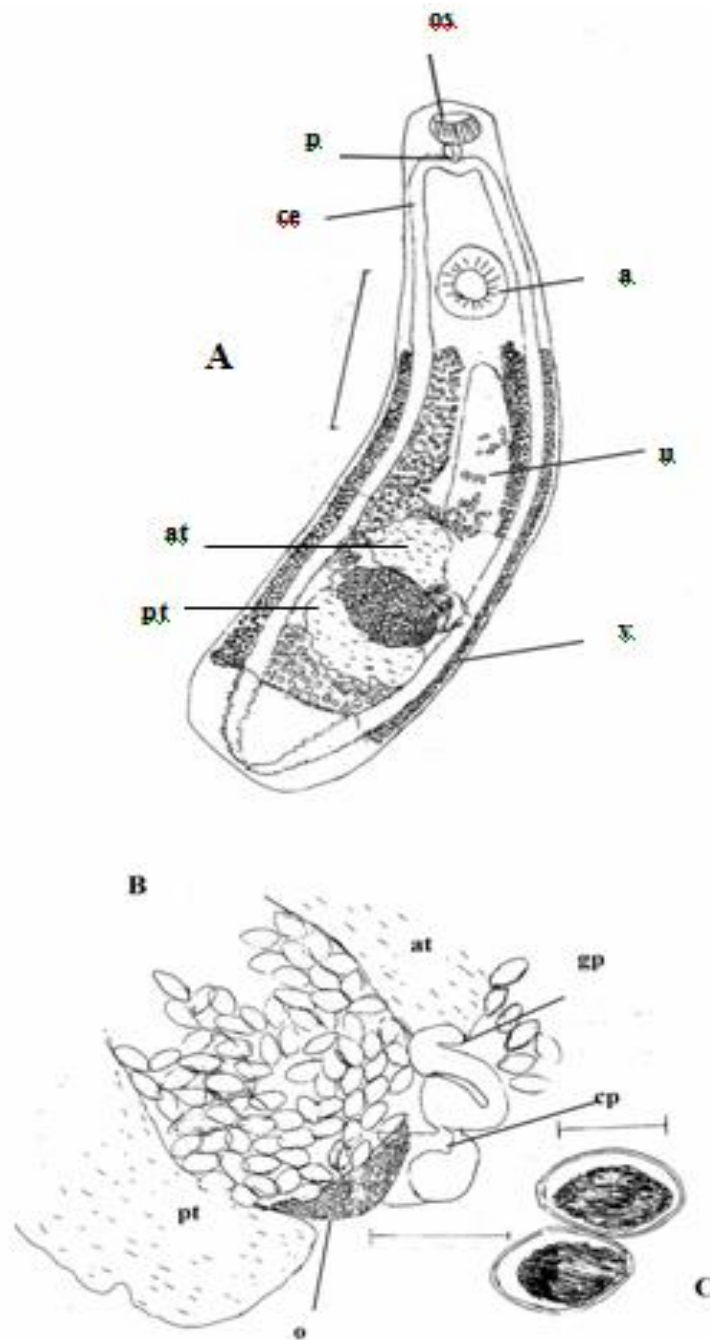


Fig.(3): *Clinostomum* sp. dorsal view, A: whole body, B: reproductive systym, C: egg from uterus. acetabulum (a), anterior testis (at), caecum (ce), cirrus pouch(cp), genital pore(gp), ovary (o), oral sucker (os), pharynx (p), posterior testis (pt), uterus (u), vitellaria (v). Scale bar: A=1mm, B=450 $\mu$ m, C=90 $\mu$ m.

Mattews and Cribb (1998) disagree with fall most or all species in the single species *C. complanatum* and gave five reasons for having long and confused taxonomic history. The first reason was many of species of *Clinostomum* were very similar to each other and differ in only relatively minor characters. The second many species have been described inadequately, the third many were described in the absence of knowledge of other species, the fourth many were described from metacercariae rather than from gravid adults, and the last was single species was even described from a cercaria. Matthews and Cribb (1998) reexamined the Australian materials previously fall as synonyms of *C. complanatum* with new specimens and used more than 30 characters, they found four valid species including one new species and *C. complanatum*.

Lo *et al.* (1982) offered 21 species in the genus which infect different fishes in the world as metacercarial stage, including two species *C. complanatum* and *C. marginatum* (Rud., 1819), they concluded that two species were different based on position of genital pore and first intermediate host and geographical distribution (*Radix auricularia* in old world in compared to *Planorbella* of North America for *C. marginatus* ), However, the two species were us as single species by

most researchers, but validity of both species verified again by Dzikowski *et al.* (2004) by differences in Ribosomal DNA , when confirm are separate species furthermore morphological characters and first intermediate hosts by impossible miracidium of *C. complanatum* to infect the snail *Planorbella* sp. (first intermediate host of *C. marginatum*). Fortunately all present materials have genital pore situated distal in cirrus sac and the results confirmed previous investigation about stability of this character in the single species.

In Iraq no study related to records or description of adult of *Clinostomum*, Al-Mayah (1994) recorded immature specimens of *C. phalacrocoracis* Dubios, 1931 from proventriculus of pygmy cormorant in Al-Sweeb Marsh North-east of Basrash province. Metacercariae of *C. complanatum* were recorded from 16 species of freshwater fishes in different parts of Iraq (Al-Saadi, 2007).

Present materials agreed in description and morphological characters with that of the same species in Matthews and Cribb (1998) from piscivorous birds of Australia and considered belong to it. This is the first record and description of parasite in Iraq and *Ardea cinerea* and *Ardeola ralloides* regarded as new hosts record.

## 2- *Clinostomum dasi* Bhalerao, 1942

Bhalerao(1942) described this parasite as metacercaria based on single specimen from stinging catfish *Saccobranchus fossilis* (= *Heteropneustes fossilis*) in freshwater of India, and it was distinguished from other species by position of the gonads in the middle third of the body and the place of connection of uterus canal with uterus sac. Ukoli(1966) place this species with 14 others which described as larval stage as synonyms with *C. complanatum*. Pandey (1966) redescribed and confirmed the validity of the species from fish as metacercaria and adult stage from buccal cavity of *Bubulcus ibis* and *Ardeola grayii* in India, Jain and Chandra (1977) and Nama (1980) were recorded this species as metacercariae from *H. fossilis* in fresh water of India.

Present species was similar to the previous one in presence of constriction at acetabulum level and position of anterior testis turn to the left from median longitudinal axis of ventral view and relatively position of ovary and cirrus sac to testes, but this species differ from previous one by small length, position of acetabulum in the body ( in the middle part of first third of body in compared with posterior part of first third of body in the former), gonad position in the body (anterior testis in the mid of middle third of body and posterior

testis mostly in mid third of body in compared to anterior testis in the posterior part of middle third of body and the posterior testis in the posterior third of body in the former), nature of caeca diverticula (highly just posterior acetabulum in compared to slightly posterior the testes and smoothly in the test of caeca in the former), ovary shape (hemi circle in compared to globular in the former), position of cirrus sac to ovary (diagonal in compared to vertical in the former), distribution of vitellaria (start just posterior third of acetabulum level in compared to behind acetabulum in the former).

Metacercaria of this species was recorded from only two fish hosts in Iraq, Ali *et al.*(1986) recorded this parasite from subcutaneous of muscle and peritoneum of *H. fossilis* from Diyala river East mid of Iraq, consequence recoded this parasite from the same fish in the mid and south of Iraq (Ali *et al.*, 1987a,b; Mohammad, 1989; and Ali, 2001) and from *Barbus luteus* in Baghdad (Al-Nasiri, 2000).

Description and morphological characters of our specimen agree with that of the same species in Pandey (1966) and was considered belong to it. This is the first record and description of parasite in Iraq and *Botaurus stellaris* as regarded as new host record in Iraq at least.

### 3- *Clinostomum* sp.

Ukoli (1966) was created key to 13 valid species in the genus *Clinostomum* parasitized birds in different part of the world. Matthews and Cribb(1998) added three species from birds of Australia.

present species similar to *C. complanatum* Rud., 1814 in size of body, suckers position, testes position, cirrus sac position and relatively in diverticula of caeca, but present species differ from the former in absence of constriction at acetabulum level, shape of ovary ( crescent in compared to oval), and place of genital pore (close to posterior corner of anterior testis in compared to median part of anterior testis) and in the position of anterior testis to centre longitudinal axis ( in the center in compared to turn of the testis to the left in the former species) and in the shape of posterior testis.

By having present species genital pore close to corner of anterior testis similar to that in *C. tilapiae* Ukoli, 1966, but differ from it by position of it in centre of longitudinal axis in compared to turn it to the left in *C. tilapiae*, and in shape of ovary (crescent in compared to folded global) and length of body (8-9 mm in compared to 5 mm in the *C. tilapiae*) and in the extended of vitellaria well distance behind acetabulum in compared to just behind acetabulum in *C. tilapiae*.

our species differ from *C.*

*phalacrocoracis* by position of gonad in the body (anterior testis in middle third of body and posterior testis in the posterior third of body in compared to both testes situated in posterior third of body) and in the shape of anterior testis (triangle in compared to fan-shape) and ovary shape (crescent in compared to globular) and in the vitellaria extended.

By having crescent-like ovary and elapsed with cirrus sac in present species which were not found in any known species (16 valid species in the genus), also combination of other diagnostic characteristics above in the present species dissimilar to that in other species, hence the description and recorded this species considered the first in the world and both *A. cinerea* and *I. minutus* considered new hosts record, according to above differences between present species and others probably this one new species.

The double infection by *C. complanatum* with *Clinostomum* sp. in *A. cinerea* and *A. ralloides* similar to that *C. complanatum* and *C. hornum* Nocoll, 1914 from Australian bittern and *C. complanatum* and *C. wilsoni* Matthews and Cribb, 1998 from intermediate egret in Australia (Matthews and Cribb, 1998), especially with low specialization of some final bird hosts to digenean trematodes (Robert and Janovy, 1996). Also some fishes such as *B. luteus* and *H. fossilis* infected with two species of

*Clinostomum* (Al-Nasiri, 2000 and Ali, 2001) take more chance to infect the final bird host with double infection.

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## تسجيل أول لثلاثة أنواع من المثقوبات جنس *Clinostomum* Leidy, 1856 من بعض الطيور الآكلة للأسماك في هور الحمار - جنوب العراق

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### الخلاصة

وصفت ثلاثة أنواع من المثقوبات العائدة للجنس *Clinostomum* Leidy, 1856 من ثلاثة أنواع مختلفة من الطيور الآكلة للأسماك فسجل النوع *C. complanatum* Leidy, 1856 من طير مالك الحزين الرمادي *Ardea cinerea* وطير الواق الأبيض الصغير *Ardeola ralloides*، النوع *C. dasi* Bhalerao, 1942 من طير الواق *Botaurus stellaris* والنوع *Clinostomum* sp. من طير مالك الحزين الرمادي وطير الواق الأبيض الصغير التي جمعت من هور الحمار جنوب العراق خلال الفترة الممتدة من كانون الأول - شباط 2004 و كانون الأول 2005. جميع الطفيليات تسجل وتوصف لأول مرة في العراق.

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