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## Isolate and Identify of *Contracaecum* spp. Nematodes from Some Aquatic Bird Species by Using Light Microscope and Scanning Electron Microscope

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### Abstract:

Ninety six aquatic birds were collected and examined for infection with *Contracaecum* spp. nematodes in Al-Sanaf marsh, which is located in Thi-Qar province / Southern Iraq, between March to December 2018, The nematodes are fixed in hot 70% ethanol, the lactophenol and glycerine used to clear the internal features and examined by light microscope. Some samples examined by scanning electron microscope (SEM) a Leo1450vp and the images were captured digitally. The current study showed that 48 out of 96 (50%) aquatic birds were infected with *Contracaecum* nematodes, *Contracaecum microcephalum* isolated from four aquatic bird species: *Nycticorax nycticorax*, *Egretta garzetta*, *Ardea purpurea* and *Pelecanus onocrotalus*, while *Contracaecum multipapilatum* isolated from two aquatic bird species *E. garzetta* and *Ardeola ralloides*, *Contracaecum rudolphii* isolated from *Phalacrocorax carbo* only.

**Key words:** *Contracaecum*, Aquatic birds, Al-Sanaf marsh, Southern Iraq.

### Introduction:

The Nematodes related to the genus *Contracaecum* (Ascaridoidea: Anisakidae) includes approximately 100 species with globally distributed, they used crustaceans and fish as their intermediate hosts and the fish-eating birds and marine mammals as definitive hosts (Anderson 2000).

The severe infection of *Contracaecum* in fish affected their health, these nematodes reduce fish production by affecting the normal physiology and if left uncontrolled, it can result in mass mortalities or in some cases, can be served as a source of infection for human and other vertebrates that consumed fish (Ayotunde *et al.*, 2007). The third larval stages of *Contracaecum* become L4 in the stomach of the definitive host, both larvae and adults may affect the final host health negatively (Martins *et al.*, 2005 ; Kanarek and Bohdanowicz, 2009). Aquatic birds are abundant in southern marshes of Iraq and they act as final host of *Contracaecum* nematodes, the studies about *contracaecum* nematodes of aquatic birds in marshes of Thi-qar province Iraqi were very limited. Only two studies reported this nematodes in aquatic birds of Thi-Qar province, *Contracaecum ovale* isolated from *Ardea cinerea* and *C. rudolphi* from *Phalacrocorax pygmaeus* in Al-Hammar marsh by Al-Kinanny (2013), while Mohammad (2014) isolated *Contracaecum*

spp. from eight aquatic bird species: *A. ralloides*, *E. garzetta*, *N. nycticorax*, *Larus genei*, *Ph. carbo*, *Bubulcus ibis*, *A. purpurea*, *P. onocrotalus*.

The identification of *Contracaecum* spp. from various host groups attracted attention of scientists in different geographical parts of the world. therefore, the present study aimed to isolate and identify the species of *Contracaecum* nematodes from different aquatic bird hosts by using light microscope and scanning electron microscope to document additional data about these nematodes and their aquatic bird hosts.

### Material and methods:

A total of 96 adult specimens of aquatic birds were examined for the presence of *Contracaecum* nematodes during the period from March to December 2018, these birds including: (1) bird belonged to *Ph. carbo* ,(25) birds belonged to *N. nycticorax*, (23) birds belonged to *E. garzetta*, , (3) birds belonged to *A. purpurea*, (43) birds belong to Squoacco Heron *A. ralloides* and (1) bird of white Pelicans *P. onocrolalus*. The birds were caught from Al-Sanaf marsh located in the western north part of Al-Hammar marsh eastern south of Thi-Qar province. This marsh's area is about 250 km<sup>2</sup> and used by many aquatic birds such as fish eating-birds as important rural fisheries resources (Mohammed, 2013).

The nematodes are fixed in hot 70% ethanol. The hot fixative causes them to straighten out making them easier to study. This procedure has no effect on dead specimens. Nematodes are usually stored in 5% glycerine in 70% ethanol following fixation rather than 70% ethanol, the lactophenol and glycerine used to clear the internal features, then specimens examined by using light microscope with divided ocular lens, drawing by camera lucida and photographed by digital camera, identified according to Yamaguti (1961), Shamsi *et al.* (2008 and 2009).

Some samples examined by scanning electron microscope (SEM), dehydrated in ethanol, placed in analytical grade ethanol for at least 1h and then transferred into hexamethyldisilazane–ethanol solutions in which the amount of hexamethyldisilazane was gradually raised to 100%. After evaporation of the hexamethyldisilazane (Shamsi *et al.*, 2008), specimens were placed on a stub, coated with gold and examined in a Leo1450vp scanning electron microscope at Ferdowsi University, Mashhad, Iran, and the images were captured digitally.

### Results and discussion:

Three species of *Contracaecum* were isolated from aquatic birds: *C. microcephalum* isolated from four aquatic bird species: *N. nycticorax*, *E. garzetta*, *A. purpurea* and *P. onocrolalus*, while *C. multipapilatum* isolated from two aquatic bird species *E. garzetta* and *A. ralloides*, *C. rudolphii* isolated from *Ph. carbo* only, (table 1.).

**Table 1. The infection with *Contracaecum* spp. according to bird species.**

Aquatic bird family	Bird species	No. birds infected with <i>C. microcephalum</i>	No. birds infected with <i>C. multipapilatum</i>	No. birds infected with <i>C. rudolphii</i>
Phalacrocoracidae	<i>Ph. carbo</i>	-	-	1
Ardeidae	<i>N. nycticorax</i>	19	-	-
	<i>E. garzetta</i>	12	5	-
	<i>A. purpurea</i>	1	-	-
	<i>A. ralloides</i>	-	23	-
Pelecanidae	<i>P. onocrolalus</i>	1	-	-

The current study revealed that 48 out of 96 (50%) aquatic birds were infected with *Contracaecum* nematodes, high prevalence of infection was recorded in *Ph. carbo* and *P. onocrolalus* 100% , while the low prevalence was in *A. purpurea* 33%. (table 2.).

**Table 2. The prevalence and mean intensity of aquatic birds infected with *Contracaecum* spp.**

Bird species	Examined No.	Infected No.	<i>Contracaecum</i> spp.	%	Mean of intensity
<i>Ph. carbo</i>	1	1	<i>C. rudolphii</i>	100.00	22
<i>N. nycticorax</i>	25	19	<i>C. microcephalum</i>	76.00	4.7
<i>E. garzetta</i>	23	1	<i>C. microcephalum</i>	4.34	12
		2	<i>C. multipapilatum</i>	8.69	5
<i>A. purpurea</i>	3	1	<i>C. microcephalum</i>	33.33	1
<i>A. ralloides</i>	43	23	<i>C. multipapilatum</i>	53.48	3
<i>P. onocrolalus</i>	1	1	<i>C. microcephalum</i>	100.00	30
Total	96	48	<i>Contracaecum</i> spp.	50.00	12.95

Mohammad (2014) reported that the high prevalence and mean intensity of infection were occurred in *P. carbo* infected with *Contracaecum* nematodes. In Basrah province Al-Hadithi and Abdullah (1991) noted *C. ovale* and *C. microcephalum* from *A. purpurea*, Awad *et al.* (1994) isolated *C. spiculigerum*; *C. microcephalum* and *C. multipapillatum* from *Ph. pygmyeus* and *C. microcephalum* from *E. garzetta*. Ali (2008) showed that the aquatic birds were infected with three species of *Contracaecum* included: *C. microcephalum*, *C. multipapillatum* and *C. ovale*

***Contracaecum rudolphii* (Hartwich, 1964):**

Males and females of *C. rudolphii* were isolated from *Ph. carbo* birds with prevalence (100%) and mean of intensity (22). Dziekonska-Rynko and Rokicki (2008) recorded *C. rudolphii* in the glandular stomach of *P. carbo* collected from north-eastern Poland with high prevalence 100.00% and high intensity reached 102.46. Al-Moussawi and Mohammad (2011) reported 91.7% of the cormorant, *P. carbo* in Baghdad area were infected with *C. rudolphii* collected from the proventriculus and ventriculus with intensity 78.66 worms per host.

The body of adult *C. rudolphii* covered with thick, transparent and striated cuticle with transverse folds. It is yellowish, coiled, tapering at each end of the body.

**Males:** Total body length 19.00-22.84 (22.56) mm, width 0.78-0.88 (0.81)mm. This species with three round labia: one dorsal and two subventral with hook-shaped interlabia between them , the interlabia approximately two thirds of height of labia.

The excretory pore at base of interlabium, each labia seem equal in size and radially digitate surface pattern with strong depression. The labia with two auricles and two pyriform papillae. The papillae of the dorsal labium seem to be unequal in size resulted from the fusion of the two original papillae. Diameter across

lips 0.20-0.14 (0.16) mm, the position of nerve ring to the anterior end 0.41-0.50 (0.45) mm, deirids 0.50-0.59 (0.53)mm, esophagus length 3.20-3.70 (3.30)mm long, 13%-16% (14%) to the body length, ventricular appendix 0.94-1.10 (1.00)mm long, 29%-31% (30%) to the esophagus length, Intestinal caecum 2.10-2.90 (2.50)mm long, 65%-78% (75%) to esophagus length, intestinal caecum long about two to third of ventricular appendix long, the right spicule long 5.40-6.80 (5.70)mm, 25%-28%(27%) to total body length, left spicule 5.80-6.80 (5.70)mm, 25%-30%(27%) to total body length, Precloacal, papillae 28-36(32) pairs, Post-cloacal papillae 6 pairs, the tail long 0.16-0.20 (0.18)mm, 0.74%-0.84% (0.80%) to total body length, the tail end curved and with conical shape. Fig. (1- 3).

### Females:

Total body length 17.60-28.40 (23.00)mm, width 0.62-0.98(0.77)mm, Diameter across lips 0.16-0.20(0.18)mm, Nerve ring 0.45-0.55(0.50)mm from anterior end.

Deirids 0.53-0.70 (0.60)mm from anterior end, esophagus long 3.00-4.00 (3.40)mm, 14%-17% (15%) to total body length, ventricular appendix 0.80-1.20 (1.00)mm, 26%-30%(28%) to esophagus length, Intestinal caecum long 2.50-3.00(2.70)mm, 75%-83% (79%) to esophagus length, tail long 0.23-0.38 (0.35)mm, 1.30%-1.80% (1.50%) to total body length, vulva 8.40-11.70 (9.20)mm from the anterior end, 41%-49%(42%) to total body length, ova long 58 - 66 (60)  $\mu$ m, width 49-50 (51)  $\mu$ m.

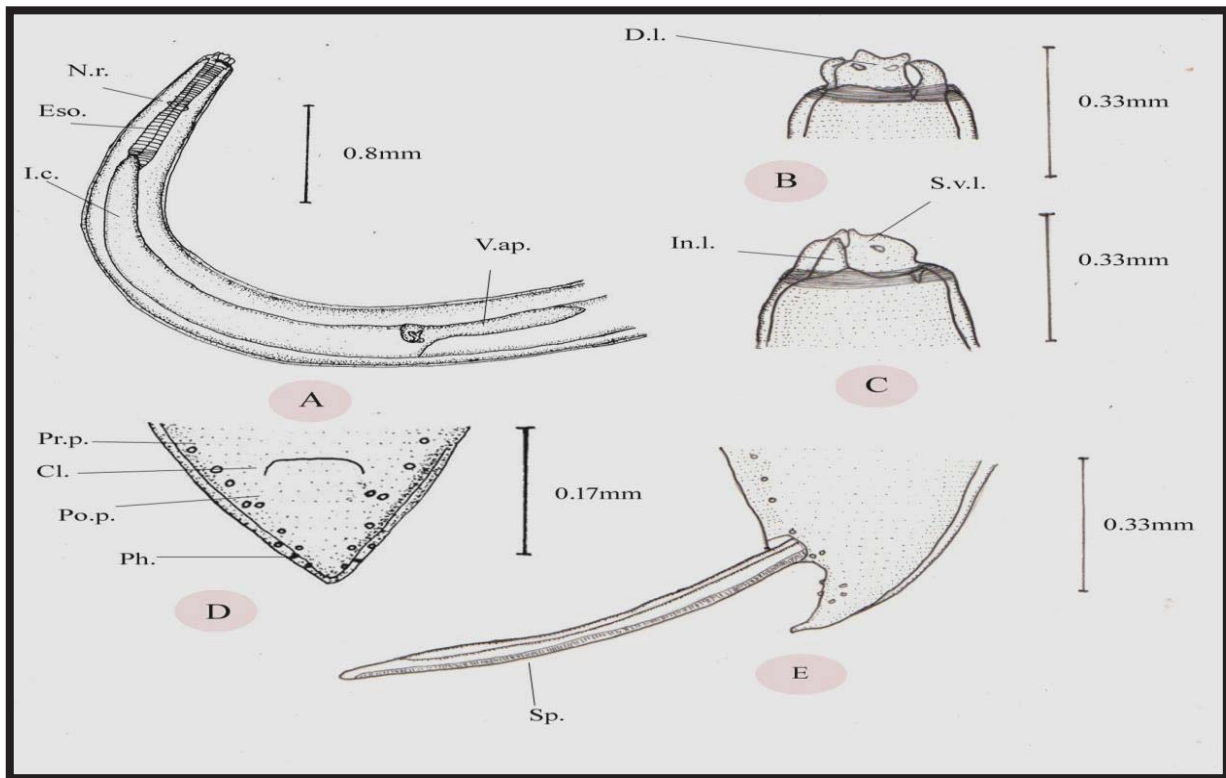


Fig. 1. *Contracaecum rudolphii* from *Ph.carbo*: A anterior end, Band C views of lips, D posterior end of female, E posterior end of male. (camera lucida).



Fig. 2. *Contracaecum rudolphii* from *Ph.carbo*: A and B views of lips, C posterior end in male.

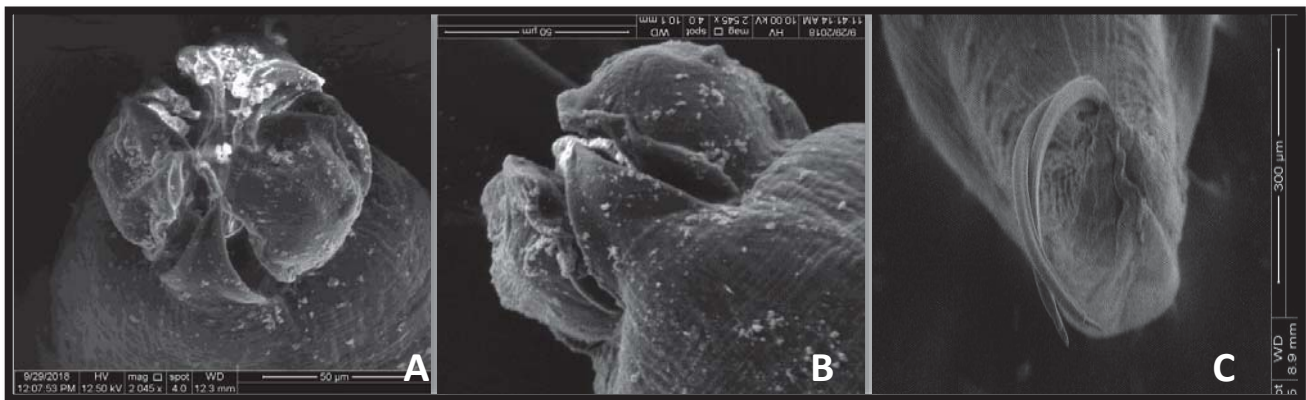


Fig. 3. Scanning electron micrographs of *Contracaecum rudolphii* from *Ph.carbo*: A and B views of lips, C posterior end in male.

This nematodes were isolated from the same host by Al-Kinanny (2013) with prevalence 90% and mean of intensity 3.5 in Al-Hamar marsh southern Thi-Qar province, Al-Moussawi and Mohammad (2011) recorded this nematode species from *Ph. carbo* with prevalence 91.7% and mean of intensity 78.66 in Baghdad, the dimensions of the male nematode corresponded with the results of present study except in number of the Post-cloacal papillae.

Previous study by Al-Moussawi (2017) in Baghdad provided the morphological description of *C. rudolphii* collected from the cormorant *Ph. carbo* by using (SEM) showing the important structures considers as a characteristic feature of this species which corresponded with the current study except the cephalic collar unclear. The features and measurements of *C. rudolphii* in present study found corresponds, more or less, with the description of the worm and scanning electron micrographs given by Shamsi *et al.* (2009).

#### ***Contracaecum microcephalum* (Rudolphi, 1809):**

This nematodes were isolated from four aquatic birds in current study: *N. nycticorax* with prevalence 76% and mean of intensity 4.7, *E. garzettawith* prevalence 4.34%% and mean of intensity 12, *A. purpurea* with prevalence 33.33% and mean of intensity 1, *P. onocrolalus* with prevalence 100% and mean of intensity 30.

This species with three equal, large, thick, round lips, these lips longer than wide, one dorsal and two lateral, sub-ventral in location, with three radial grooves derived from deep depression and characterized with

anterolateral flanges, the dorsal labium with two large, ellipsoidal cephalic papillae, both subventral labium of this species with one ellipsoidal cephalic papillae.

Interlabia and labia had the same height, the interlabia with triangular shape and simple tips, the internal part of interlabia free and curved. The cuticle behind the head finely annulated and this nematode with deep indentation separated the collar from the rest of the body, male and female with Centrids at midbody region. Table (3, 4), Fig (4-6).

**Table 3. The measurements of *C. microcephalum* males in different aquatic bird hosts.**

Character in mm	<i>N. nycticorax</i>	<i>E. garzetta</i>	<i>A. purpurea</i>	<i>P. oncotralus</i>
Total body	22,80 (28-18,00)	18,40 (23-10)	22,00	(22,00-16,80) 18,48
Width	0,71 (0,77-0,62)	0,61 (0,70-0,52)	0,77	(0,90-0,50) 0,68
Labia	19,40 (22-0,17)	0,18 (0,23-0,14)	0,18	(0,18-0,14) 0,16
Nerve ring	0,08 (0,62-0,00)	0,04 (0,60-0,49)	0,74	(0,00-0,40) 0,44
Deirids	0,60 (0,71-0,61)	0,09 (0,66-0,03)	0,84	(0,64-0,46) 0,04
Esophagus	3,70 (4,20-3,20)	3,62 (4,80-3,00)	0,70	(3,00-2,20) 2,60
Eso./total body	%16	%19	%25	%13
ventricular appendix	1,18 (1,60-0,90)	1,22 (1,40-1,10)	1,60	(0,10-0,88) 0,99
ventricular appendix/Eso.	%31	%33	%28	%38
intestinal cecum	2,84 (3,30-2,40)	2,44 (2,90-2,22)	4,00	(2,20-1,00) 1,92
intestinal cecum/Eso.	%76	%67	%79	%73
Tail long	0,27 (0,31-0,20)	0,20 (0,31-0,22)	0,28	(0,20-0,16) 0,18
Tail/total body	%1,2	%1,3	%1.3	%1
Right spicule	3,44 (4,30-2,80)	3,17 (3,00-2,90)	2,96	(3,40-2,20) 2,90
Right spicule/total body	%10	%17	%13	%10
Left spicule	4,63 (4,00-2,80)	3,38 (3,90-3,00)	3,24	(3,60-2,40) 3,20
Left spicule/total body	%16	%18	%14	%17
Pre-cloacal papillae	29 (31-20)	29 (32-26)	31 pairs	22 (28-18)
Post-cloacal papillae	6 pairs	6 pairs	6 pairs	6 pairs

Table. 4. The measurements of *C. microcephalum* females in different aquatic bird hosts.

Character in mm	<i>N. nycticorax</i>	<i>E. garzetta</i>	<i>P. onocrotalus</i>
Total body	20,10 (27-22)	22,0 (23-22)	-21,20 23,30 (26,60)
Width	0,70 (0,88-0,68)	(0,76-0,70) 0,73	(0,92-0,76) 0,80
Labia	0,19 (0,22-0,16)	(0,19-0,17) 0,18	0,18
Nerve ring	0,08 (0,63-0,00)	(0,00-0,49) 0,02	(0,08-0,42) 0,00
Deirids	0,71 (0,78-0,60)	(0,63-0,60) 0,62	(0,64-0,00) 0,08
Esophagus	3,63 (3,80-3,30)	(3,80-3,64) 3,72	(3,70-3,10) 3,30
Eso./total body	%14	%16	%14
ventricular appendix	1,20 (1,33-0,90)	(1,30-1,28) 1,31	(1,20-0,90) 1,06
ventricular appendix/Eso.	%32	%30	%32
intestinal cecum	2,62 (2,93-2,40)	(3,00-2,90) 2,90	(2,80-2,40) 2,00
intestinal cecum/Eso.	%72	%79	%70
Tail long	0,36 (0,40-0,32)	(0,31-0,27) 0,29	(0,38-0,40) 0,39
Tail/total body	%1,4	%1,3	%1,6
Vulva to anterior end	10,70 (12-9)	(10,00-9,00) 10,00	(7,60-7,00) 7,28
Vulva/total body length	%42	%44	%31
Egg long	61 (59-64) μm	61 (60-62) μm	60 (58-62) μm
Egg width	54.80 (54-56) μm	55 (54-56) μm	54 (53-55) μm

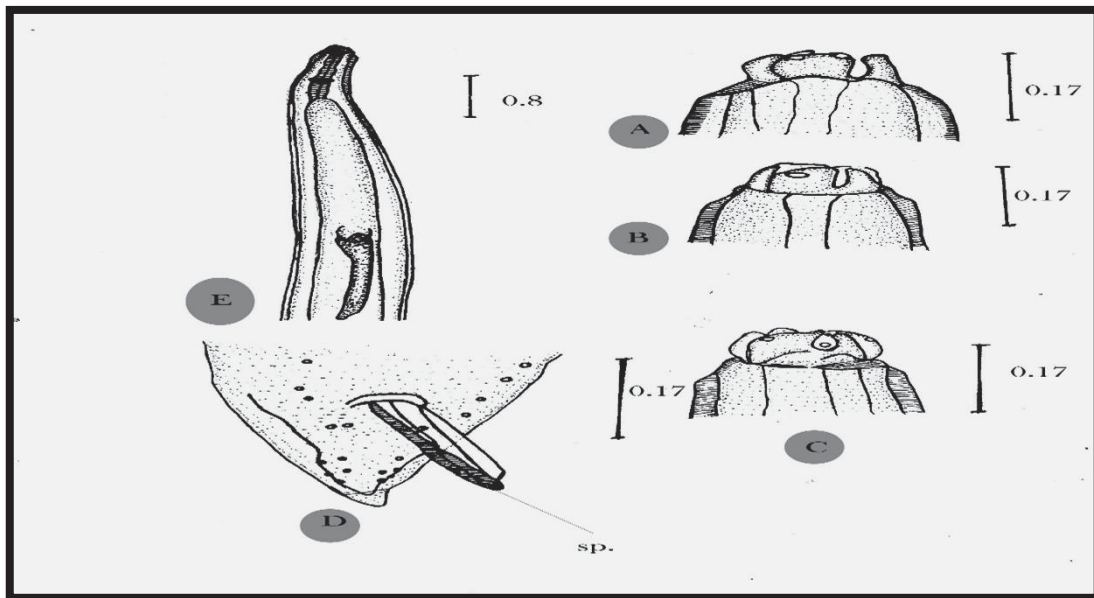


Fig. 4. *Contracaecum microcephalum*, A, B and C views of lips and interlabia, D posterior end in male, E anterior end. (camera lucida).



Fig. 5. *Contracaecum microcephalum*, A anterior end, B posterior end in male.

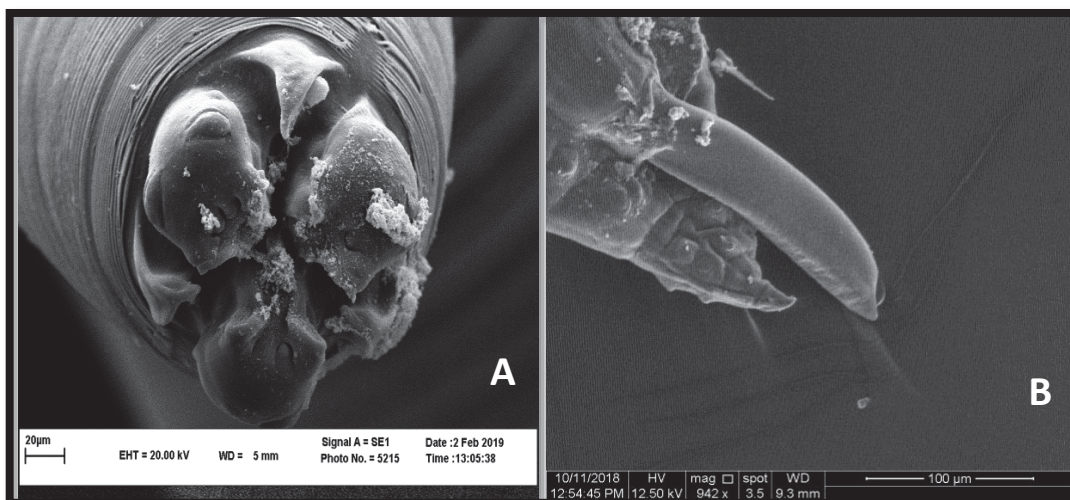


Fig. 6. Scanning electron micrographs of *C. microcephalum* A views of lips, B posterior end in male.



*C. microcephalum* nematodes were isolated for the first time in Iraq by Al-Hadithi and Habish (1977) from *A. purpurea* birds in Basrah province, this nematodes also isolated from the same bird species in Basrah province by Al-Jaborae *et al.* (1992), while Swadi (2013) reported *C. microcephalum* in *A. ralloides*, *Bubulcus ibis* and *Halcyon smyrnensis* in Shatt AL-Arab coast.

The description of *C. microcephalum* in current study correspond with the study of Barson and Marshall (2004) described *C. microcephalum* isolated from *Anhinga melanogaster* by using scanning electron microscope. The dimensions of *C. microcephalum* in current study found corresponds, more or less, with the description of the nematodes and scanning electron micrographs given by Shamsi *et al.* (2009) for this species isolated from *Pilcanus melanoleucos*.

***Contracecum multipapillatum* (Drashe 1882):**

This nematode species isolated in current study from two aquatic bird species *A. ralloides* with prevalence 53% and mean of intensity 3.00, *E. garzetta* with prevalence 8.69% and mean of intensity 5.00. The anterior end provided with three large, fleshy lips, one dorsal and two lateral sub-ventral in position, the dorsal lip with pair of cephalic papillae, while the ventral lips provided with only one cephalic papillae located at the mid level of lip. Lips without dentigerous ridges, Interlabia and libia had the same height, the interlabia with triangular shape, collar separated the anterior end from the rest of the body and found at the base of lips, ventriculus reduced and globular with solid posterior appendix , it about 1/3-1/4 to intestinal caecum long. Table (5, 6), Fig. (7-9).

**Table. 5. The measurements of *C. multipapillatum* males in different aquatic bird hosts.**

Character in mm	<i>A. ralloides</i>	<i>E. garzetta</i>
Total body	14,00 (16,00-13,10)	22,60 (27-19,00)
Width	0,66 (0,68-0,60)	0,70 (0,83-0,60)
Labia	0,14 (0,18-0,13)	0,17 (0,20-0,10)
Nerve ring	0,41 (0,44-0,40)	0,00 (0,02-0,47)
Deirids	0,00 (0,60-0,40)	0,07 (0,09-0,00)
Esophagus	2,40 (3,00-1,90)	3,64 (4,90-2,40)
Eso./total body	%16	%16
ventricular appendix	0,86 (1,10-0,70)	1,24 (1,80-0,80)
ventricular appendix/Eso.	%30	%31
intestinal cecum	1,90 (2,60-1,40)	2,94 (4,20-1,80)
intestinal cecum/Eso.	%80	%80
Tail long	0,14 (0,19-0,12)	0,19 (0,20-0,18)
Tail/total body	%1	%0,8
Right spicule	2,10 (2,70-1,60)	1,77 (2,00-1,40)
Right spicule/total body	%10	%7
Left spicule	2,70 (3,70-1,70)	1,80 (2,00-1,46)
Left spicule/total body	%18	%7
Pre-cloacal papillae	31 (38-27)	33 (34-32)
Post-cloacal papillae	10 Paris	10 Paris

Table. 6. The measurements of *C. multipapillatum* females in different aquatic bird hosts.

Character in mm	<i>A. ralloides</i>	<i>E. garzetta</i>
Total body	17,30 (14,70-19,10)	24,82 (20-29)
Width	0,88 (0,70-1,00)	0,79 (0,60-0,90)
Labia	0,16 (0,10-0,20)	0,18 (0,10-0,25)
Nerve ring	0,40 (0,20-0,60)	0,51 (0,30-0,70)
Deirids	0,57 (0,40-0,70)	0,73 (0,50-0,90)
Esophagus	3,00 (2,30-3,70)	3,00 (2,00-4,00)
Eso./total body	%17	%12
ventricular appendix	1,10 (0,80-1,40)	1,10 (0,70-1,50)
ventricular appendix/Eso.	%36	%30
intestinal cecum	2,20 (1,50-2,90)	2,70 (1,70-3,70)
intestinal cecum/Eso.	%73	%76
Tail long	0,27 (0,10-0,40)	0,33 (0,20-0,40)
Tail/total body	%1,60	%1,3
Vulva to anterior end	7,70 (6,00-9,40)	10,00 (8,00-12,00)
Vulva/total body length	%44	%40
Egg long	μm 72 (50-90)	μm 93 (70-110)
Egg width	μm 50 (30-70)	μm 48 (30-60)

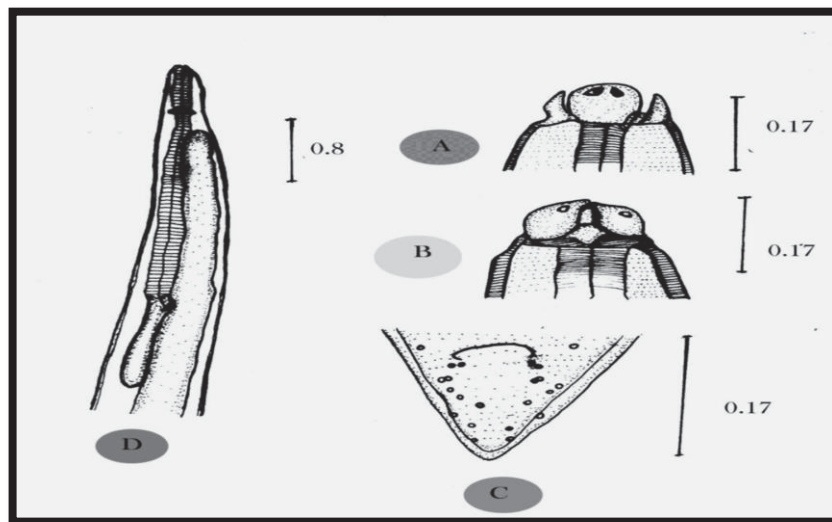


Fig. 7. *C. multipapillatum* A and B views the libs and interlabia, C male posterior end, D anterior end. (Camera lucida).

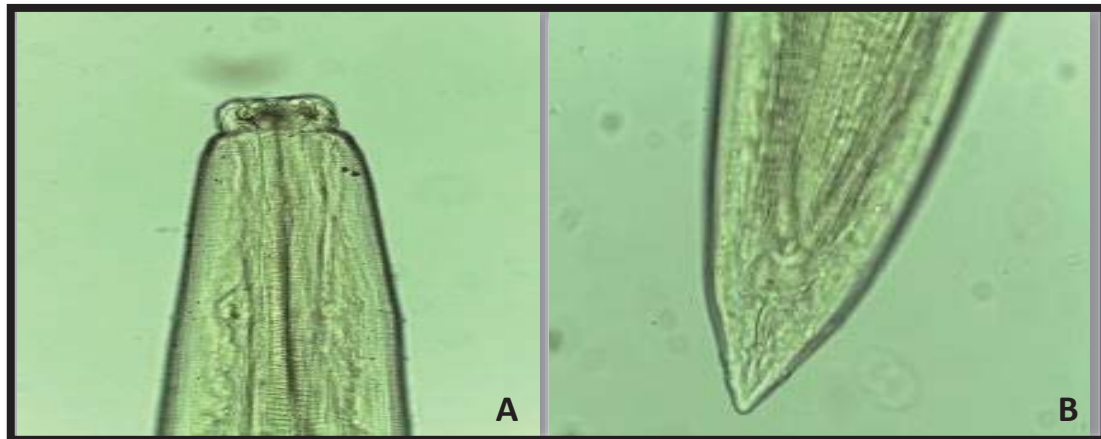


Fig. 8. *C. multipapillatum* A anterior end, B male posterior end.

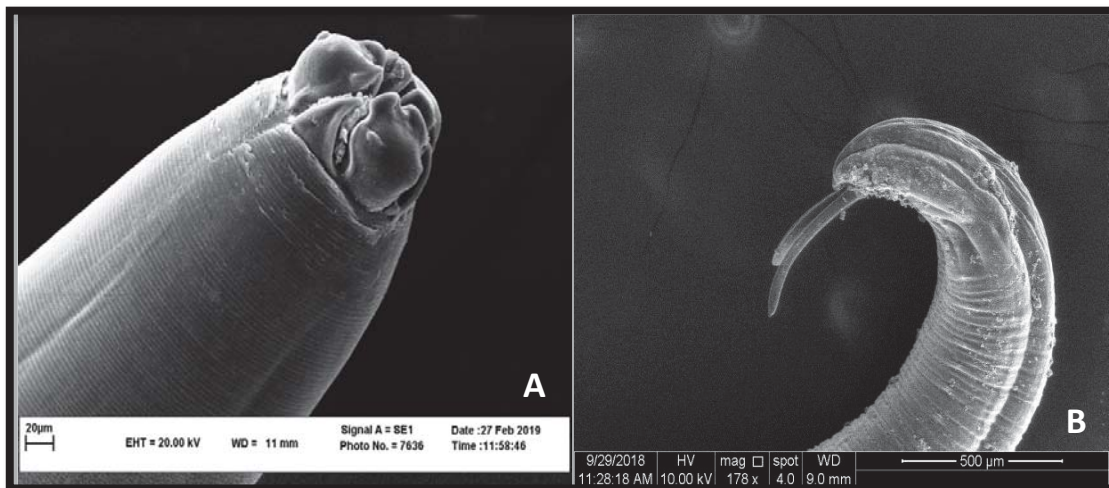


Fig. 9. Scanning electron micrographs of *C. multipapillatum* A views of lips, B posterior end in male.

In Argentina *C. multipapillatum* were isolated from *Egretta alba* by Navone *et al.* (2000), D'amelio *et al.* (2007) described *C. multipapillatum* from *Pelecanus occidentalis* by using ITS gene in USA, while Girisgin *et al.* (2012) isolated this nematodes from *Pelecanus crispus* in Turkey.

In current study the *C. multipapillatum* males smaller than the females, males isolated from *A. ralloides* were shorter, with less lips diameter and longer spicules when compared with males isolated from *E. garzetta*, The females of this species which isolated from two hosts in current study corresponded in most important features.

The most characteristics feature of *C. mutipapllatum* in current study correspond with the study of Shamsi *et al.* (2008) especially the samples isolated from *E. garzetta*.

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