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Insights at morphological features of *Contracaecum rudolphii* Hartwich, 1964 (Nematoda: Anisakidae) as revealed by scanning electron microscope (SEM)

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Abstract

The present study provides morphological description of adults (male and female) of *Contracaecum rudolphii* Hartwich, 1964 collected from the proventriculus and ventriculus of the cormorant *Phalacrocorax carbo* (Linnaeus, 1758) in Iraq using scanning electron microscopy (SEM) showing the structures of cephalic extremity of the nematode, labium, interlabium, papillae in the dorsal labium, the morphology of caudal papillae in male, phasmids, the shape of the tip of spicule which considers as a characteristic feature of this species. A comparison with previous studies is provided.

Keywords: *Phalacrocorax carbo*, nematode, *Contracaecum rudolphii*, morphology, scanning electron microscopy

1. Introduction

Phalacrocorax carbo is one of birds wintering in suitable water bodies in Iraq^[1]. It consumes fishes, young fishes and fingerlings of fish farms in Iraq^[2], and serves as a final host for some helminthes. Few papers have been carried out on parasitic helminthes of *P. carbo* in Iraq, including Abed^[3] who isolated three larvae of *Contracaecum* (*Contracaecum* sp.1 *Contracaecum* sp.2 *Contracaecum* sp.3); Khudhaier *et al.*^[4] who reported the cestode *Haploparaxis crassirostris*; Al-Moussawi and Mohammad^[5] recorded *Contracaecum rudolphii*; Mohammad and Al-Moussawi^[6] isolated the trematode *Paryphostomum testitriofolium* and the cestode *Paradilepis scolecina*; later, Al-Tameemi^[7] reported the cestode *P. scolecina*.

Nematodes of the genus *Contracaecum* are found mainly in piscivorous birds^[8]. Adults of *C. rudolphii* found parasitizing cormorants mainly and the paratenic intermediate hosts for this nematode are aquatic invertebrates which harbor the infective third-stage larvae (mainly copepods, crustaceans and insects) and fishes, such as *Cyprinus carpio*, *Rutilus rutilus*, *Tinca tinca*^[9, 10].

C. rudolphii (which recorded as *C. spiculigerum*) by Yorke and Maplestone^[11], is a cosmopolitan anisakid nematode. This nematode is the most important parasite of cormorants, it was described for the first time from the oesophagus and stomach of *Phalacrocorax carbo* by Hartwich at 1964^[10]. The final hosts for species of the genus *Contracaecum* are fish eating birds^[12], genus *Phalacrocorax* mainly^[13].

Morphological studies have been done on species belonging to the genus *Contracaecum* with scanning electron microscopy (SEM), including Fagerholm and Gibson^[14]; Fagerholm^[15],¹⁶ and^{17]}; Amato *et al.*^[18], Kim *et al.*^[8] and Moravec and Scholz^[10].

In Iraq, few studies have been done on the adults of some species of the genus *Contracaecum* from avian hosts using light microscopy^[3, 5, 19, 20, 21, 22, 23, 24]. Nevertheless, there is no study had been done for any species belongs to the genus *Contracaecum* using scanning electron microscopy. So, the present study was conducted to define the morphological features for *Contracaecum rudolphii* found in *Phalacrocorax carbo* from Baghdad area with scanning electron microscopy (SEM).

2. Materials and Methods

Sixteen adult nematodes were collected from the proventriculus and ventriculus of the cormorant *P. carbo* were collected from Baghdad city, they found morphologically corresponding to *Contracaecum rudolphii* following (Yorke and Maplestone^[11] and Amato *et al.*^[18]).

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The specimens of the nematode were prepared for scanning electron microscope through graduate steps following El-Kabbany [25], they washed carefully in distilled water to which a few drops of detergent solution had been added to dislodge any attached debris, fixed in a solution containing 4% aqueous glutaraldehyde and 1% osmium tetroxide [OSO₄] for 2 hours., washed in distilled water, dehydrated in acetone and dried using carbon dioxide [Co₂] in a critical point dryer mounted on aluminium stubs and sputter coated with gold. Specimens were examined with a Jeol ISM5300 scanning electron microscope at Mansora University, Faculty of Science; electron microscopy center.

3. Result and Discussion

Sixteen adult specimens (8 males and 8 females) of *C. rudolphii* were found in the proventriculus and ventriculus of the cormorant. *P.carbo* was whitish and medium sized nematode. Figure (1A, B, C and D) showed anterior part of the body with cephalic structures, small head; fine annulations posterior to head; cephalic collar; excretory pore; 3 bifid interlabia; 3 labia which seem equal in size, 2 auricles, 2 papillae on each labia and the excretory pore at base of interlabium. The two papillae in the dorsal labium are unequal in size, this correspond well with Amato *et al.* [18] and Hartwich [26] who mentioned to the presence of the two pairs of papillae in the dorsal labium as a result of the fusion of the two original papillae. (Fig. 2A, B, C and D) showed the posterior part of body of the male of *Contraecaecum rudolphii*, precloacal papillae, cloacal opening with 2 spicules. The small phasmids found laterally between 2 postcloacal papillae. The conical tail of the worm end with a sub pointed tip. Spicule slender, alate, pointed apically. the shape of the tip of spicule correspond well with that of Moravec and Scholz [10], who stated that Hartwich [26] considered the shape of the distal end of spicule as a characteristic feature to differ this species from the three congeners species, *C. microcephalum*; *C. micropapillatum* (Stossich, 1890) and *C. variegatum* (Rudolphi, 1809).

Fig. 3 show the posterior extremity of female body, phasmids locates near the tip of the tail.

In some features such as anterior part of body, the structures of cephalic extremity of the nematode, the number and

distribution pattern and morphology of caudal papillae in males, phasmids, tip of the spicule in male, and the shape of the posterior end of *C. rudolphii* in the present study, found corresponds, more or less, with the description of the worm and scanning electron micrographs given by Amato *et al.* [18] and Moravec and Scholz [10], and with the redescription of the nematode given by Li *et al.* [27].

Using scanning electron microscopy in morphological studies for some ascaridoid nematodes had been presented in earlier studies included, Fagerholm [15, 17] who discussed the pattern distribution of the caudal papillae in *C. rudolphii* and 10 another *Contraecaecum* species at different regions of the world, and he pointed out the importance of studying the caudal papillae and papillae-like structures in the males and gave a diagrammatic presentation and a special nomenclature for their number and distribution; Baruš *et al.* [12] showed the distribution of the caudal papillae of *C. rudolphii* as well as the morphology of the labia and interlabia; Abollo *et al.* [28] who discussed the caudal papillae distribution in *Contraecaecum rudolphii* and *C. septentrionale* found in the cormorant *Phalacrocorax aristotelis* and the study of Amato *et al.* [18] who presented a revision for some morphological studies which were conducted earlier and they discussed the pattern distribution of the caudal papillae in males of *C. rudolphii* from the cormorant *Phalacrocorax brasilianus* with examining the morphology of the labia, interlabia. In addition, morphological and genetic studies have been carried out which indicated that *C. rudolphii* represents a complex of sibling species [10, 29, 30, 31, 32]. Garbin *et al.* [32] had been demonstrated that *C. rudolphii* is a complex of five sibling species which designated as *C. rudolphii* A, *C. rudolphii* B, *C. rudolphii* C, *C. rudolphii* D and *C. rudolphii* E., whereas Li *et al.* [27] mentioned that there are six sibling species within the *C. rudolphii* complex according to molecular studies. *C. rudolphii* B found parasitizing the cormorant *Phalacrocorax carbo sinensis* which present in freshwater environments in central Europe [30]. The molecular study presented by Szostakowska and Fagerholm [31] identified *C. rudolphii* B in freshwater fishes in Poland; this sibling species was recorded then in cormorants in freshwater sites, whereas *C. rudolphii* A isolated from cormorants occurred in brackish water regions in Finland and Poland [33].

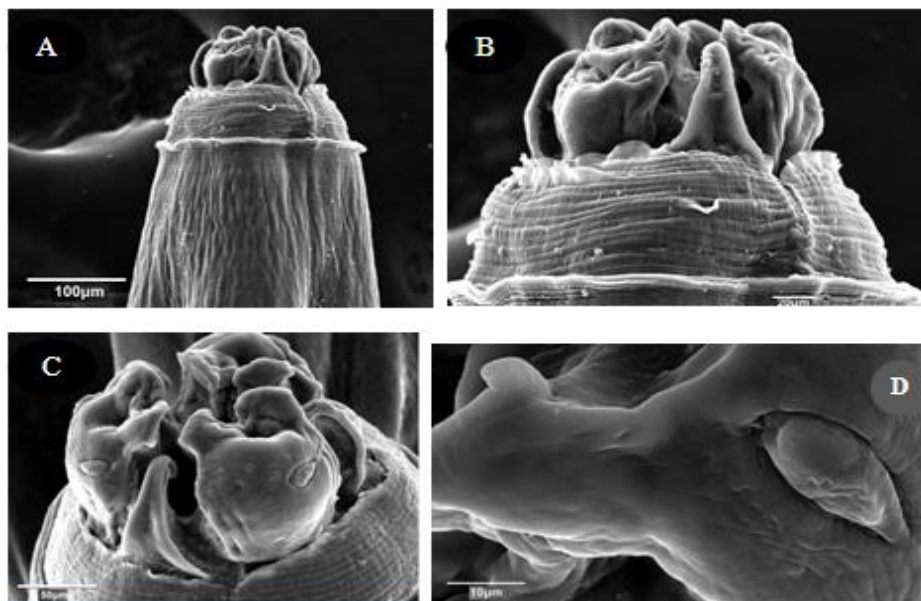


Fig 1: Scanning electron micrographs of lateral view of male of *Contraecaecum rudolphii* Hartwich, 1964 from *Phalacrocorax carbo*: A. anterior part of the body; B. head, cephalic collar; C. cephalic extremity; D. magnified view for a side of the labium, ovate papillae, auricle.

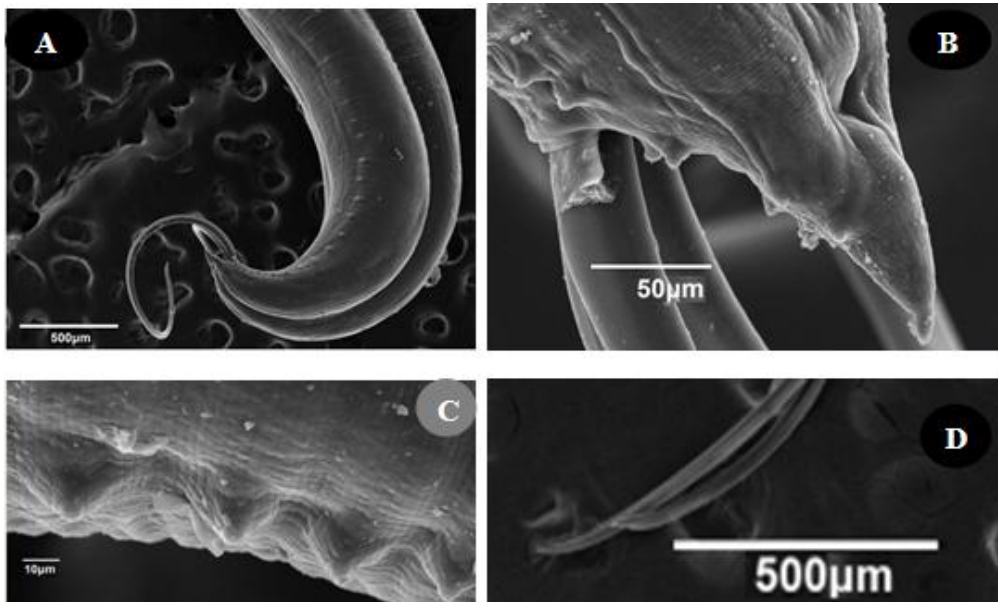


Fig 2: Scanning electron micrographs of male of *C. rudolphii* : A. preloacal papillae, spicules ,tail; B. posterior part of the body, cloaca, 2 spicules , tail, postcloacal papillae, lateral phasmid; C. magnified view of preloacal papillae; D. magnified view of the distal end of spicule.

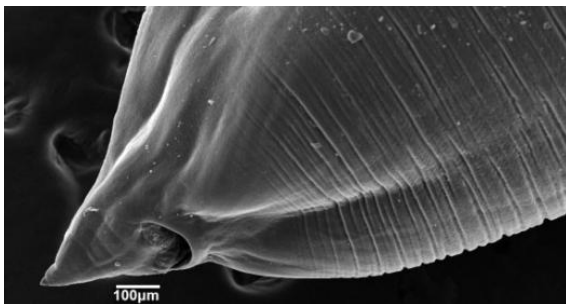


Fig 3: Scanning electron micrograph for posterior part of female of *C. rudolphii*

4. Conclusion

It is appropriate to mention here that since *C. rudolphii* in the present study was collected from *Phalacrocorax carbo*, which consumes freshwater fishes in Iraq [2, 34], it may be *C. rudolphii* B.

To determine the existence of the complex of sibling species of *Contraecaecum* in fish eating birds of Iraq (species of the genus *Phalacrocorax* in particular), so, future genetic studies are suggested to be done.

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