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Research Article

Helminth Community, with Special Reference to the Acanthocephalans, *Sphaerirostris* sp. (Polymorphida) and *Mediorhynchus* sp. (Gigantorhynchidae), in the Shorebird, *Calidris temminckii* (Aves: Charadriiformes), in Qena, Upper Egypt

[1]Nermean M. Hussein and [2]Khaled Mohamed El-Dakhly

[1]Department of Zoology, Faculty of Science, South Valley University, Qena, Egypt
[2]Department of Parasitology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt

Abstract

Background and Objective: In Egypt, scarce literature posing the existence of helminths, particularly acanthocephalans, where migratory/wading birds are available. The present study aimed to investigate the helminths, particularly acanthocephalans, in necropsied wading birds, *Calidris temminckii*, in Qena province, Upper Egypt. **Materials and Methods:** Necropsy of 22 captured shore birds (7 males and 15 females), *Calidris temminckii*, aged more than 2 years alongside the Nile River in Qena, Egypt was done. The recovered acanthocephalans were routinely processed and stained with potassium alum carmine and/or cleared in lactophenol and mounted on glycerol-jelly. Moreover, drawing and scanning electron microscopy were applied. **Results:** Two species of acanthocephalans were recovered from the intestinal tract; *Sphaerirostris* sp. Golvan 1956 (Centrorhynchidae, Van Cleave, 1916) in eight (2 males and 6 females) (36.36%) out of 22 birds and *Mediorhynchus* sp. Van Cleave, 1916 (Gigantorhynchidae, Hamann, 1892) in five (22.73%) birds (females). Based on drawings with camera lucida, light microscopy and scanning electron microscopy, acanthocephalan species were diagnosed. Moreover, one parasitized female bird by the acanthocephalan, *Mediorhynchus* sp. was co-infected with the digeneans, *Neodiplostomum* sp. and *Paryphostomum radiatum*, the tapeworm, *Paradilepsis scolecina* and the spirurid nematode, *Streptocara* sp. Meanwhile, all acanthocephalans-infected birds were parasitized by *Paryphostomum radiatum* and *Paradilepsis scolecina*. Further studies are needed to highlight on more information regarding taxonomic and genotypic variations among acanthocephalan species in migratory birds and other wildlife in Egypt. **Conclusion:** Two species of acanthocephalans, *Sphaerirostris* sp. (Polymorphida) and *Mediorhynchus* sp. (Gigantorhynchida), were found parasitizing the intestinal tract of necropsied shore birds, *Calidris temminckii* in Qena province, Upper Egypt. Other helminths, *Neodiplostomum* sp., *Paryphostomum radiatum* (digeneans), *Paradilepsis scolecina* (cestodes) and *Streptocara* sp. (nematodes) were also found. To the best of our knowledge, the current study is the first report of the occurrence of acanthocephalan species in the shore birds, *Calidris temminckii* in Egypt.

Key words: *Calidris temminckii*, *Sphaerirostris* sp., *Mediorhynchus* sp., acanthocephalans, helminth community

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Corresponding Author: Khaled Mohamed El-Dakhly, Department of Parasitology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt Tel: +2 01100103010 Fax: +2 082 2327982

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Acanthocephalans, thorny-headed worms, are helminths commonly endoparasitic in the intestine of piscine and avian definitive hosts, with the developmental stages occurring in the gut of terrestrial arthropod intermediate hosts[1-3]. Paratenic hosts, such as reptiles and amphibians, are often involved when definitive hosts are predators[4]. The classification of acanthocephalans with a complete identification and description of their classes, families and genera is previously known[5-8]. A key characteristic for the phylum is the armed eversible proboscis with numerous hooks[4].

Golvan (1956) erected *Sphaerirostris* as a subgenus of *Centrorhynchus* Luhe, 1911 including 21 species. Recently, *Sphaerirostris* is obviously a genus of serious taxonomic revision, which will undoubtedly lead to the creation of more synonymies[9]. Diagnosis of such genus mainly based on the number and distribution pattern of hooks on the proboscis[3].

Most acanthocephalan species, including other members of *Mediorhynchus*, are not nearly so host specific and infect a variety of species having similar diets[10]. Except for *M. centurorum* which penetrates deeply into the intestinal wall of vertebrate hosts causing nodular formation into the coelom, other species of *Mediorhynchus* have not been reported to cause similar lesions[11]. Globally, few reports erected species of *Mediorhynchus*; among those Ward[12] revealed *Mediorhynchus kuntzi* (new species) from the stone curlew (*Burhinus senegalensis*) (Aves: Charadriiformes) in Wadi Nassim, Isna, Qena province, Schmidt and Kuntz[13] released seven species of *Mediorhynchus* in South America[14] and Amin *et al*.[8] assigned *M. africanus* from the helmeted Guinea fowl, *Numida meleagris* Linn., in South Africa. The taxonomic status of *Mediorhynchus* species within the Archiacanthocephala, is no less confused when evaluated from the perspective of molecular studies. Phylogenetic reconstructions using 18S ribosomal RNA genes[15], 18S and 28S ribosomal RNA genes[16], mitochondrial cytochrome c oxidase subunit 1[17], the whole mitochondria[18-19], locate *Mediorhynchus* and Archiacanthocephala in varying relationships.

Coming from Europe and Asia, *Calidris temminckii* is strongly migratory, wintering at freshwater sites in tropical Africa. During its traveling, such birds may feed on lots of arthropod intermediate hosts as well as paratenic hosts with a great opportunity to complete the life cycle of acanthocephalans. In Egypt, helminthological investigations of migratory, wading and predatory birds are scarce due to irregular or occasional hunting seasons. Therefore, the current study aimed to describe helminth fauna in the captured migratory birds, *Calidris temminckii*, from Qena province, Egypt, with special reference to the detailed morphometric and scanning electron microscopy of the recovered acanthocephalans. Such concern might be useful in understanding the biology of zoonotic helminths, as these birds may serve as paratenic/definitive hosts for those helminths.

MATERIALS AND METHODS

The study is based on specimens from 13 aquatic birds, *Calidris temminckii*, captured from an urban area near the Nile river in Qena (coordinates: 26°10'12"N 32°43'38"E), Upper Egypt during the period from November, 2016-January, 2017. Birds were obtained from different localities alongside the Nile river. Apparently, birds did not suffer from injury, diseases or other abnormalities. They were taken to the Laboratory of the Faculty of Science, South Valley University for parasitological investigations.

The necropsied birds (n = 22, 7 males and 15 females) were dissected and the intestinal tract of each bird was longitudinally incised and carefully examined for the presence of acanthocephalans. The intestinal contents were collected and poured in clean petri dishes.

The recovered intact acanthocephalans were carefully selected, washed in tap water, fixed in FAA solution (a mixture of 92 mL 70% ethyl alcohol+5 mL formalin+3 mL acetic acid) for 24 h and cleared in lactophenol and mounted with glycerol-jelly[3]. Some specimens were stained with potassium alum carmine after fixation[20]. All specimens were drawn with camera lucida and photographed using a digital microscope (Leica microsystems, CH-9435 Heerbrugg, Ec3, Singapore).

For scanning electron microscopy (SEM), specimens immersed in 3% gluteraldehyde buffered with 0.1 M phosphate buffer (pH 4) at room temperature for 2-4 h. Washing tissues with 0.1 M phosphate buffer (pH 7.2) 3 times, 10 min each. Specimens were post fixed with 1-2% osmium tetroxide in 0.1 M phosphate buffer (pH 7.2) in a light container for 2-4 h at room temperature. Washing tissues with 0.1 M phosphate buffer (pH 7.2) 3 times, 10 min each. Dehydration in a graded ethanol/acetone solutions (30, 50, 70, 80, 90, 96 and 100% for 5-15 min, each grade). A double folded dehydration with 100% ethanol or acetone (15-30 min, for each) was done. Finally, samples were mounted on a copper sluds, gold coated and observed with a

scanning electron microscope[21]. Digital images of the structures were obtained using JEOL JSM-5500 LV scanning electron microscope (JEOL, Japan) equipped with Oxford energy dispersive X-ray microanalyzer EDX system with link software and model 6587 X-ray detector (Oxford, England).

RESULTS

Necropsy of 22 shore birds (7 males and 15 females), *Calidris temminckii*, revealed the presence of two species of acanthocephalans, *Sphaerirostris* sp. Golvan 1956 (Centrorhynchidae, Van Cleave, 1916) (8/22; 36.36%) and *Mediorhynchus* sp. Van Cleave, 1916 (Gigantorhynchidae, Hamann, 1892) (5/22; 22.73%). It has been found that 2 males were found to be infected with *Sphaerirostris* sp. and 6 females were parasitized. Moreover, another 5 female birds were infected with the acanthocephalan, *Mediorhynchus* sp. The latter was not recorded in male birds. No co-infection with the two acanthocephalan species was detected. Identification of the recovered acanthocephalans based on drawing with camera lucida, light microscopy and scanning electron microscopy.

It is worthy to mention that 1 female bird was co-infected by the acanthocephalan, *Mediorhynchus* sp., the digeneans, *Neodiplostomum* sp. and *Paryphostomum radiatum*, the tapeworm, *Paradilepsis scolecina* and the spirurid nematode, *Streptocara* sp. Meanwhile, all acanthocephalans-infected birds were parasitized by *Paryphostomum radiatum* and *Paradilepsis scolecina* (Table 1).

- ***Sphaerirostris* sp.:** (Fig. 1-3)
- **Host:** The wading bird, *Calidris temminckii*
- **Locality:** Shorelines of Nile river, Qena, Egypt

Diagnosis: The body was smooth. The male measured 8-9 mm and the female measured 9.5-10.2 mm. The proboscis was ovoid measuring 0.70-0.78 mm in males and 0.85-0.96 mm in females. The anterior part of the proboscis measured 0.34-0.36×0.30-0.32 mm in males and 0.37-0.42×0.35-0.36 mm in females, while the posterior part measured 0.41-0.43×0.30-0.33 mm in males and 0.57-0.58×0.4-0.41 mm in females. The trunk was non-spinose, short, spindle-shaped and measured 7.30-8.20 mm in males and 8.65-9.20 mm in females. Lemnisci were longer than proboscis sheath measured (2.51-2.56 mm in length and 0.26-0.27 mm in width. The number of hook rows was 21-24 and the number of hooks in each longitudinal row was 12-17. The longest hook in the first row measured 27-30×6.5-6.8 µm but the hooks in the last raw measured 16-18×8-9 µm. The length of neck was 0.39-0.46 mm. Eggs were oval measuring 47-49×23-24 µm. In males, the bursa evened and measured 1.5-1.6×0.87- 0.91 mm. Based on the revealed criteria, the acanthocephalan was identified as *Sphaerirostris* sp.

- ***Mediorhynchus* sp.:** (Fig. 4-6)
- **Host:** The wading bird, *Calidris temminckii*
- **Locality:** Shorelines of Nile river, Qena, Egypt

Diagnosis: The male measured 48.0-57×1.30-1.40 mm, while the female was 60.0-68.0×1.70-2.0 mm. The

Table 1: Occurrence of mixed infection of acanthocephalans-infected shore birds, *Calidris temminckii*, from Qena, Upper Egypt

Infected bird number	Sex	Acanthocephalans recovered	Mixed infection with		
			Digeneans	Tapeworms	Nematodes
1	♂	*Sphaerirostris* sp.	*Paryphostomum radiatum*	*Paradilepsis scolecina*	-
2	♀				
3	♀				
4	♀				
5	♀				
6	♀				
7	♂				
8	♀				
9	♀	*Mediorhynchus* sp.	*Neodiplostomum* sp. *Paryphostomum radiatum*	*Paradilepsis scolecina*	*Streptocara* sp.
10	♀		*Paryphostomum radiatum*	*Paradilepsis scolecina*	-
11	♀				
12	♀				
13	♀				

Fig. 1(a-d): Light microscopy of adult *Sphaerirostris* sp. recovered from the intestine of *Calidris temminckii*, (a) Stained adult female acanthocephalan, (b) An anterior end of the worm showing proboscis armed with hooks, (c) The posterior end of adult male showing cement glands and the bursa and (d) The posterior end of the adult female

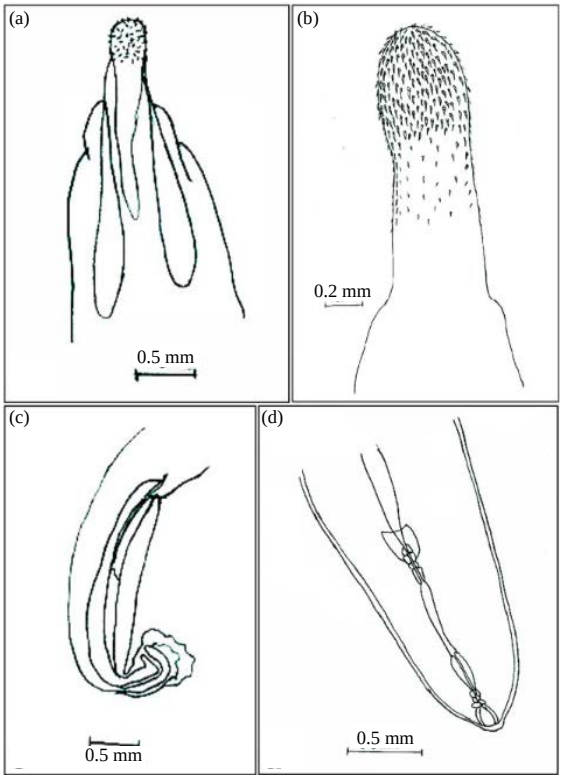

Fig. 2(a-d): A drawing of *Sphaerirostris* sp. with camera lucida, (a) The anterior end of the worm. Note the elongated lemnisci, (b) A magnified part of the proboscis with distinct rows of hooks, particularly in the anterior part, (c) The posterior end of the male acanthocephalan showing a well-developed bursa and (d) A posterior end of the female

anterior body was ovate, 2.1-2.4 mm long separated by constriction from the remaining body. The neck was very short. The number of longitudinal rows was 17-18, while the number of hooks in each row was 18-22. The proboscis measured 0.7-0.75× 0.4-0.44 mm in males and 0.88-1.0×0.4-0.5 mm in females. The trunk measured 47.3-56.25 in males and 59.2-67.0 mm in females. The longest hook length measured 32-37×6.5- 7.8 µm. Lemnisci measured 3.41-3.63×0.30- 0.32 mm. The egg was oval and measured 21-22×53-54 µm. The adult male possesses 8 nucleated pyriform cement glands. Based on the revealed criteria, the acanthocephalan was assigned as *Mediorhynchus* sp.

To the best of our knowledge, this is the first study reporting acanthocephalan species in the shore birds, *Calidris temminckii* in Egypt.

DISCUSSION

In Egypt, the distribution pattern of helminth community, particularly acanthocephalans, in migratory birds and other wildlife is still under investigation. The current study revealed the presence of two acanthocephalans, *Sphaerirostris* sp. and *Mediorhynchus* sp. in the shorebird, *Calidris temminckii* in Qena, Egypt. Authors believe that the feeding habits (flesh-eating) of the surveyed shorebirds as well as the abundance of various intermediate hosts and/or paratenic hosts encouraged the presence of these acanthocephalans.

During the past decades, little information denoted the acanthocephalans from migratory birds in Egypt. Among those, Meyer[22] described *Mediorhynchus tenuis* from *Saxicola bimaculata* and *Monticola solitarius* (Passeriformes: Turdidae); Ward[23] emerged a new species of *Centrorhynchus* from the kite, *Milvus migrans*, Ward[12] revealed *Centrorhynchus*

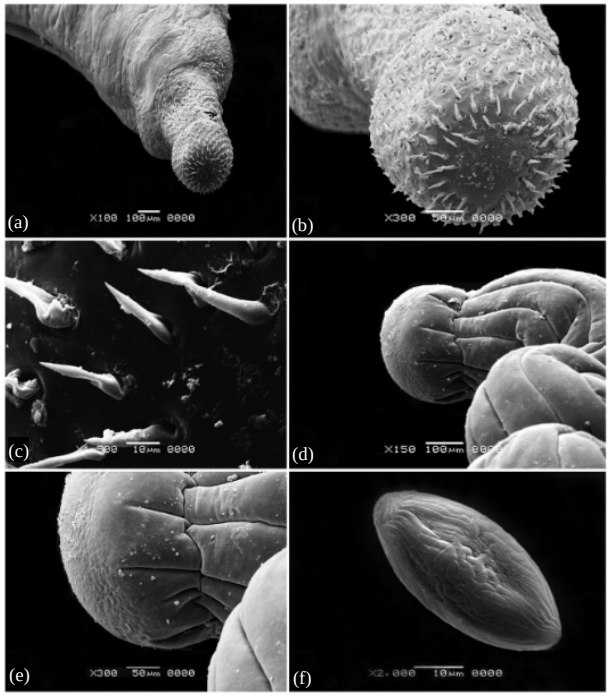

Fig. 3(a-f): Scanning electron microscopy of the acanthocephalan, *Sphaerirostris* sp., (a-c) The anterior part of the worm with its hooks, (d, e) The posterior part of adult male worm with a distinct bursa and (f) A characteristic oval-shaped egg

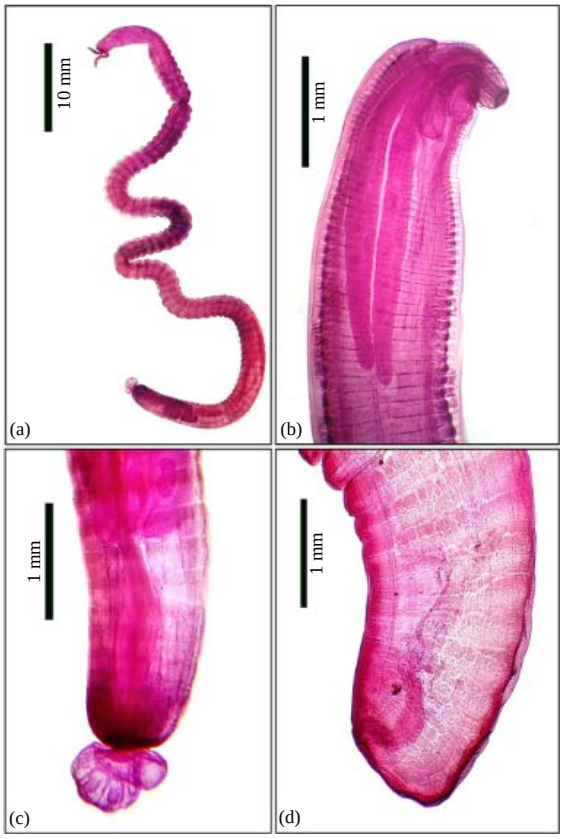

Fig. 4(a-d): Light microscopy of adult *Mediorhynchus* sp. recovered from the intestine of *Calidris temminckii* (a) Stained adult male specimen, (b) The anterior end with very long lemnisci, (c) The posterior end of the adult with a well-developed and bulged bursa and (d) The posterior end of the adult female

lancea (Westrumb, 1821), *Centrorhynchus milvus* Ward[23] and *Mediorhynchus kuntzi* (new species) from the stone curlew (*Burhinus senegalensis*) (Aves: Charadriiformes) in Wadi Nassim, Isna, Qena province as well as *Centrorhynchus corvi* (Fukui, 1929) from the plover (*Hoplopterus spinosus*) (Aves: Charadriiformes) at Lake Qarun, Fayoum province and Radwan *et al.*[24] found one acanthocephalan, *Sphaerirostris picae* Rudolphi (1819) (Centrorhynchidae) from ninety five hooded crows, *Corvus corone cornix* Linnaeus 1758 from Kafr El Sheikh province.

Flesh-eating birds, like *Calidris temminckii*, are commonly parasitized by *Sphaerirostris* species that use terrestrial isopods and insects as intermediate hosts. Moreover, snakes, frogs and lizards act as paratenic hosts successfully allowing the completion of the life cycle of such parasites. Meanwhile, species of *Mediorhynchus* are commonly found in charadriiformes and passeriformes and they need intermediate rather than paratenic hosts to complete their life cycle.

Currently, the area of study, Qena province, is a part of a large district called Upper Egypt and the areas neighbored the entire shore of the Nile river in such district is known to have several species of invertebrates as well as frogs and snakes, thus, the life cycle of *Mediorhynchus* species is encouraged. The topography of the shoreline in Qena province easily permit the existence of various crustaceans, lizards and amphibians that transmit the cystacanth to the birds, the definitive hosts.

In the present study, the digeneans, *Neodiplostomum* sp. and *Paryphostomum radiatum* were found in one female bird. The former needs snails and frogs intermediate hosts with snakes and lizards as paratenic hosts[25] and in the latter, snails and fish are intermediate hosts[26]. Moreover, Scholz *et al.*[27] mentioned that the tapeworm, *Paradilepsis scolecina*, is a common cestode in ichthyophagous birds with the main sources of infection are cyprinid fish which are preferable food sources for such birds, although crustaceans serve as the first

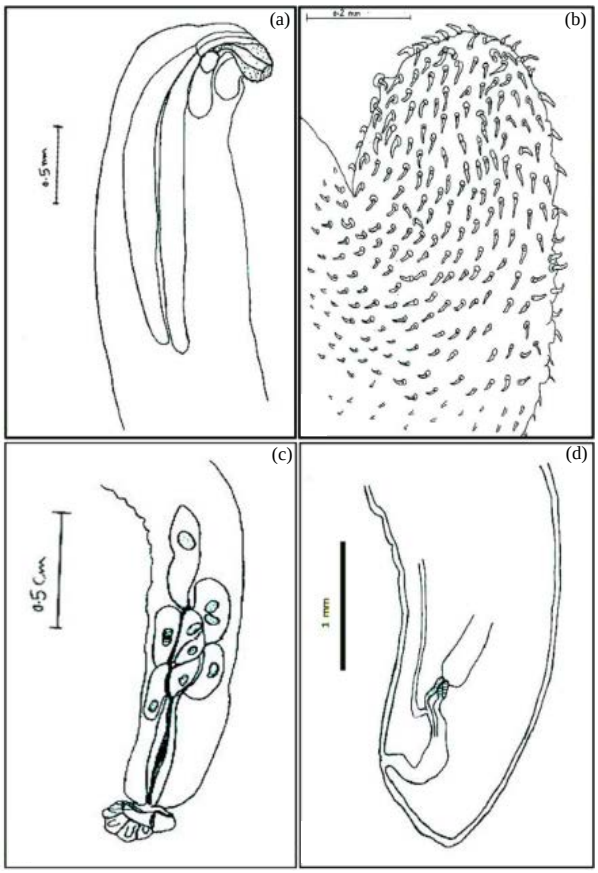

Fig. 5(a-d): A drawing of an adult *Mediorhynchus* sp., (a) The anterior end of the worm. Note the very elongated lemnisci, (b) A magnified part of the proboscis, (c) The posterior end of the male acanthocephalan showing a well-developed and bulged bursa as well as the cement glands and (d) A posterior end of the female

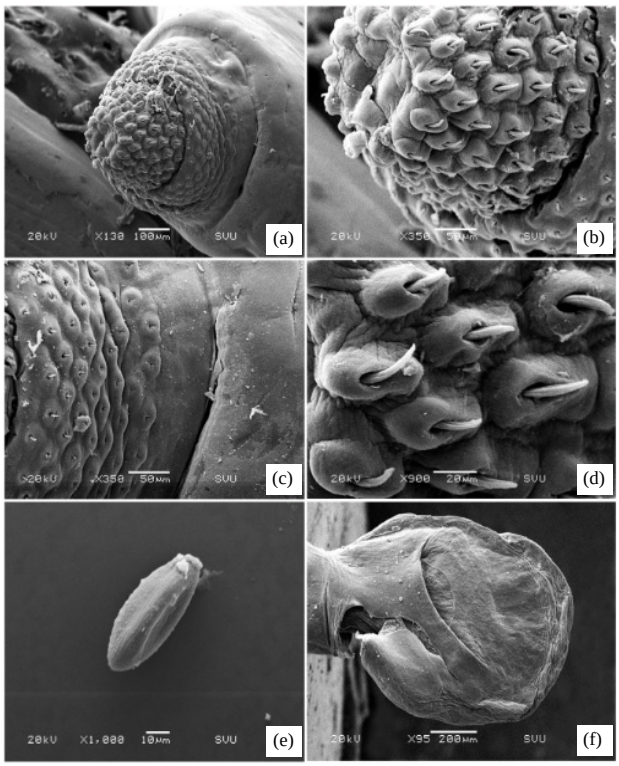

Fig. 6(a-d): Scanning electron microscopy of the acanthocephalan, *Mediorhynchus* sp., (a-d) The anterior part of the worm with its hooks, (e) An elongated egg and (f) A characteristic bulged bursa in the posterior part of the adult male

intermediate host[28]. The recovered spirurid nematode, *Streptocara* sp., requires amphipods and fish to complete the life cycle[29]. Currently, the geographical conditions alongside the Nile river in Qena province, allow the presence of various species of freshwater snails, amphipods, crustaceans and fish, therefore, the developmental stages of lots of helminths could be obtained. On the basis of this fact, the presence of *Neodiplostomum* sp., *Paryphostomum radiatum*, *Paradilepsis scolecina* and *Streptocara* sp. could be explained. On the other hand, previous literature recorded that cestodes act as a competitor for acanthocephalans sharing with the same feeding habitats and both are confined to the small intestine, providing a potential for overlapping[24,30].

CONCLUSION

It is concluded that the two species of acanthocephalans, *Sphaerirostris* sp. (Polymorphida) and *Mediorhynchus* sp. (Gigantorhynchida), were found parasitizing the intestinal tract of necropsied shore birds, *Calidris temminckii* in Qena province, Upper Egypt. Other helminths, *Neodiplostomum* sp., *Paryphostomum radiatum* (digeneans), *Paradilepsis scolecina* (cestodes) and *Streptocara* sp. (nematodes) were also found infecting the birds. To the best of our knowledge, the current study is the first report of the occurrence of acanthocephalan species in the shore birds, *Calidris temminckii* in Egypt. Due to being the number of examined birds in the current study is relatively inadequate, further expanded studies must be considered to detect the bird age and bird sex-parasite relationship as well as the histopathological alterations induced by the acanthocephalans in various species of wading birds in Egypt.

SIGNIFICANCE STATEMENTS

This study elucidated the helminth community, particularly acanthocephalans, of the shorebird, *Calidris temminckii*, in Qena, Upper Egypt emphasizing on the morphometry, distribution pattern and biology of those helminths. This study will help the parasitologists to understand the ecology of helminths in wading birds in Egypt. The close contact of these birds and fish in the same habitats highlights on the possibility of the existence of life cycles of several helminths in fish posing on the potential hazards in the consumed fish.

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