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Light and Scanning Electron Microscopy of *Sphaerirostris* sp. Nov. (Acanthocephala: Centrorhynchidae) from Hooded Crow *(Corvus corone cornix)* (Aves: Corvidae) of El-Minia Governorate, Upper Egypt

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Abstract: *Sphaerirostris* sp. nov. was obtained from the intestine of the Hooded crow (*Corvus corone cornix*) (Aves: Corvidae) from El-Minia governorate upper Egypt. The acanthocephalan was present in 6 out of 36 (16.7%) examined hosts. The intensity of infection varied from 1-4 parasites per infected bird. This species of Acanthocephala is characterized by thick-walled Body, smooth, elongated, narrowing toward posterior end with a reticular lacunar system. The specimens had 16-20 proboscis hook rows on the ovoid anterior proboscis and 14-16 spine rows on the cone-shaped posterior proboscis, each with 8-10 hooks and5-8spines per row, respectively. Proboscis receptacle is double walled; Lemnisci slightly unequal, testes in the anterior trunk; three tubular cement glands; Saefftigen's pouch and cement glands elongated; bursa is well developed and muscular. Eggs oval without polar prolongations and numerous.

Key words: Acanthocephala · Corvidae · Centrorhynchidae · El-Minia · Egypt

INTRODUCTION

Acanthocephala or the spiny-headed worms represent a group of helminths commonly found in the intestine of piscine and avian parasitic hosts [1]. A key characteristic for the group is the armed evertible proboscis with numerous hooks [2]. Commonly found in fish and birds, with more being described each year [3, 4]. *Sphaerirostris* (Golvan, [5] has been in a state of confusion due to its subgenus status to *Centrorhynchus* Luhe, 1911. Golvan [6] listed 26 species of *Sphaerirostris*, the number is questionable with more being described. Many of these were based on hook number and pattern found on the proboscis. This genus needs additional research and revision including nucleic acid (DNA) studies [7]. Some key characteristics of *Sphaerirostris* include the hook armature on the proboscis, a polydentritic lacunar system, 3 or 4, tubular cement glands and an enlarged midway proboscis. The host, hooded crow is a common resident throughout Egypt inhabiting cultivated land and open and wooded terrain in the Nile Delta and valley [8]. This species is particularly common in populated areas, especially where food resources are available in large amounts. The hooded crow is a pet characterized by a high reproductive potential [9]. The feeding habits of hooded crow include wide range of intermediate (insects, mollusks) and paratenic (small vertebrates and carrion) hosts [10] that may qualify it to harbor a wide range of parasites. Birds of the family Corvidae are among the most common species of wild birds and their free roaming in human residential areas and poultry farms, native or industrial, may be a threat to the health of other birds and, to some extent, to human beings [11]. Although the hooded crow is common and widespread occurrence in Egypt since ancient times, no comprehensive studies of its parasitic fauna has been caried out. Acanthocephala has been reported from the Hooded crow from Egypt [12, 13]. In the present study specimens belonging to a new species of *Sphaerirostris* Golvan [5] collected from the Hooded Crow from El-Minia governorate upper Egypt are described herein.

MATERIALS AND METHODS

Thirteen acanthocephalan specimens were recovered from six Crow out of Thirty six were collected from *Ashmunin* city south El-Minia governorate and examined for acanthocephalan parasites. Worms were isolated from

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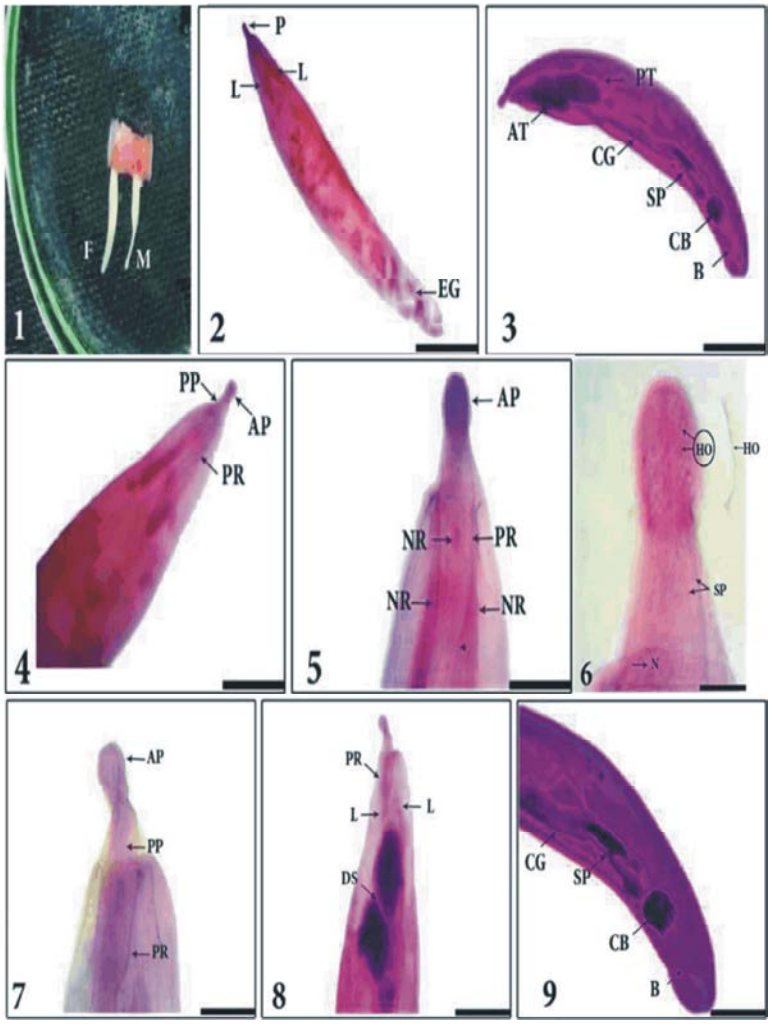
the intestine, transferred to a clean 0.9 % saline solution by using abrush and washed several times to remove any mucous or debris which is usually adhere to body surface. Acanthocephalans were relaxed in tap water, this was important for their proboscis to be fully everted from their bodies [14], then fixed in. 10% formalin. After fixation, the collected samples were washed in distilled water for 15 minutes to remove the excess fixative and then processed to staining which is carried out by using acetic acid alum carmine overnight, [15]. After staining, a differentiation step must be carried out to remove the excess stain by placing the stained worms into a dilute solution of acid alcohol (1mlHclin 99 ml 70%alcohol), it is better to carry out this process under a binocular dissecting microscope to detect the end point of differentiation. This is followed by dehydration in an ascending series of ethanol, 30%, 50%, 70%, 90%, 95% and absolute ethanol, leaving the parasites for 10-15 minutes in each grade. The specimens were then cleared in clove oil and xylene, mounted in Canada balsam, covered with cover glass and left to dry in an oven at 40°C. Morphometric measurements are given length by width in millimeters minimum and maximum values were given. For scanning electron microscopy, acanthocephalans were fixed in 3% buffered gluteraldehyde, washed in cacodylate buffer and dehydrated in an ascending alcohol series. After passing through an ascending series of the Genosolv-D, they were processed in a critical point drier "Bomer-900" with freon 13 and according to the Integrated sputter coated with goldpalladium in a Technics HummerV and examined with an Etec Autoscan at 20 kV Jeol adjusted; scanning EM.

RESULTS

The following observations and measurements are given for the acanthocephalan observed in the intestine of the hooded crow. All measurements are in mm. Of the thirteen worms observed, 8 were males and 5 females (Fig1).

Description Female: Trunk slender, gradually tapering towards posterior end Measuring 16.5±1.2 (15.76-17.24) by 3±0.7(2.33-3.54). (Fig 2). Body with reticular lacunar system with prominent nuclei. No microspores were observed through Tegment (Fig. 13). The anterior Proboscis oval (Figs 4,5,6.) narrowest at level of 8-9 hooks, Measuring 0.36±0.04 (0.33-0.40) by 0.26±0.03 (0.23- 0.30); divided by insertion into two regions, well armed with16-20 longitudinal rows of hooks each row has 8-10 hooks,. First 7-8 hooks large, with powerful roots, rest of hooks spine-shaped. The hooks measure 0.024±.004 (0.020-0.027) by 0.007±0.001 (0.006-0.009), while the spines are smaller, measuring 0.014±0.005 (0.009-0.019) by 0.004±.001 (0.002-0.005). The posterior proboscis appear conical 0.28±0.08(0.20- 0.37) by 0.34±0.05 (0.27-0.40). Neck measures 0.11±0.02 (0.08-0.10) in length. (Figs 10-12). Proboscis receptacle, double walled, elongate measuring 1.06±0.02 (1.04-1.08) by 0.29±0.08 (0.20-0.38) inserted at about middle of proboscis with oval ganglion in its mid-region. Lemnisci longer than the proboscis receptacle, the left measuring 3±0.4 (2.5-3.4) by 0.12±0.02 (0.1-0.14) and the right measuring 3.2±0.4 (2.7-3.6) by 0.15±0.05 (0.1-0.18) each with oval ganglion (Fig 4,5). Uterus long, tubular full of elongated egg balls (Fig. 2). The eggs without polar prolongations with wavy appearance on surface measuring 0.045±0.004 (0.040-0.049) by 0.016±0.002 (0.013-0.018) (Fig. 14). Gonoporesubventral, but not visible in Scan specimen (Fig. 15).

Description-Male: somewhat shorter than females, Trunk slender, gradually tapering towards posterior end, measuring 15.20±2 (13.80-16.12) by 2.8±0.4 (2.5.-3) (Fig. 3). Body with reticular lacunar system with prominent nuclei. Tegment with many microspores (Figure 20). Anterior Proboscis spherical to oval narrowest at level of 8-9 hooks, Measuring 0.300±0.040 (0.267-0.333) by 0.340±0.035 (0.300-0.373); divided by insertion into two regions, The posterior proboscis appear conical Measuring 0.192±0.008 (0.186-0.200) by 0.320±0.020 (0.300-0.333). The rows of hooks and number of hooks are similar to female specimens. (Fig. 16.). The hooks measure 0.028±0.007 (0.021-.0.033) by 0.007±0.002 (0.005-.0.009), while the spines are smaller, measuring. 015±0.002 (0.014-0.017) by 0.003±0.001 (0.002-0.005). (Fig. 18, 19). Apical view of specimen. showing an apical area also armed with spines (Fig. 17). Neck short, 0.036±0.003 (0.033-0.040) length. Proboscis receptacle, double walled, elongate measuring 0.8±0.2 (0.9-1.1) by 0.3±0.1 (0.2-0.4) inserted at about middle of proboscis with oval ganglion in its mid-region. Lemnisci longer than the proboscis receptacle, the left measuring 1.9±0.1 (1.8-2) by 0.25±0.05 (0.2-0.3) and the right measuring 2.2±0.2 (2-2.4) by 0.25±0.05 (0.2-0.3) each with oval ganglion (Figs 7,8). Testes in the anterior portion of trunk. tandem, the anterior measuring 1.5±0.3 (1.2-1.7) by 0.5±0.1 (0.6-0.7), the posterior measuring 1.5±0.2 (1.4-1.7) by 0.8±0.2 (0.6-0.9), in immature stage the distance observed between testes measure 0.08+-0.02 (0.05-0.10) (Fig. 8) Cement glands 3, measuring 3.2±0.2 (3-3.3) by 0.42±0.01 (0.40-0.43) join



Figs. 1: Photomicrographs of Whole mount Female (F) & male (M) of *Sphaerirostris.* sp. nov. collected from Crow. The worms attach with the intestinal mucosa of host.

2. Photomicrograph of Whole mount preparation of female show Proboscis (p), slightly unequal Lemnisci (L). The Figure shows elongated ovarian balls (EG), bar 3.2 mm.
3. Photomicrograph of Whole mount preparation of *S.* male shows overlap anterior (AT) & posterior (PT) testes, Cement glands (CG), Saefftigen's pouch (SP), copulatory bursa (CB) & Bursa (B), bar2.6mm
4. Photomicrograph of anterior portion of female Shows anterior (AP), Posterior (PP) Proboscis &Proboscis receptacle (PR), bar1.1mm
5. Magnification part of figure (4) Shows the oval anterior Proboscis (AP). conical posterior Proboscis (PP) double walled Proboscis receptacle (arrow) with central nerve ring (ganglion) Lemnisci each with ganglion (NR), bar 0.9 mm
6. Enlarged heavily armed proboscis of female, the hooks (HO) are arranged in longitudinal rows. The posterior part of the proboscis has spines (SP). The Figure shows the short neck (N) of female, bar0.4mm
7. Photomicrograph of anterior portion of male shows the oval anterior Proboscis (AP). conical posterior Proboscis (PP) double walled Proboscis receptacle, bar 0.5mm
8. Photomicrograph of anterior portion of male shows Proboscis receptacle (PR), Lemnisci (L) anterior (AT)& posterior (PT) testes, show distance (DS) between the testes in immature stage, bar 1.2mm
9. Magnification part of posterior end of male shows three Cement glands (CG), Saefftigen pouch (SP) serrate copulatory bursa (CB) & Bursa (B), bar 1.5mm

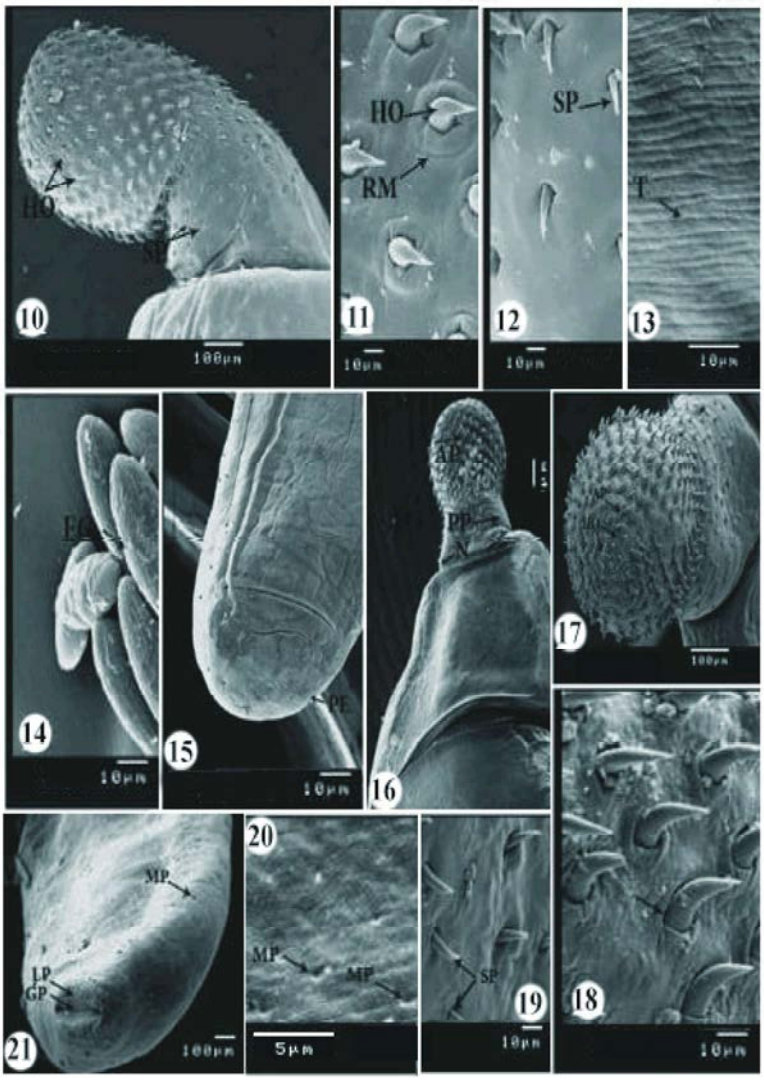


Fig. 10: Through 21 represent the result of the Scanning pictures for the Acanthocephala *S.*. sp. nov. found in Crow

Fig. 10-12: Scanning electron micrographs showing the Proboscis of an adult female shows oval anterior Proboscis (AP). conical posterior Proboscis(PP) armed with numerous hooks(HO) & Spines (SP) respectively. Proboscis & the hooks(HO) are arranged in longitudinal rows, the posterior part of the proboscis has spines (SP).

Fig. 11,12: Magnification parts of figure (10) Showing hooks surrounding with Rim (RM) and spines armed the posterior Proboscis.

Fig. 13 In tegument of female (T) No micopores were visible.

Fig. 14: Shows the oval shaped eggs (EG) which have a wavy appearance on the surface.

Fig. 15: Shows the posterior end of female (PE) The female gonopore was not visible for the samples.

Fig. 16,17: Represents the Proboscis of an adult male shows oval anterior Proboscis (AP). conical posterior Proboscis (PP) armed with numerous hooks (HO) & Spines (SP) respectively. The flat apical, covered end of Proboscis (AP). Proboscis & the hooks (HO) are arranged in longitudinal rows, the posterior part of the proboscis has spines (SP).

Fig. 18,19: Magnification parts of figure (17) Showing hooks surrounding with Rim (RM) and spines armed the posterior Proboscis.

Fig. 20: In tegument of male (T) micopores (MP) were numerous.

Fig. 21: Shows the posterior end of male The gonopore (GP), slit like Terminal & surrownding by lips (LP)

posteriorly in 2 elongate plump cement reservoirs surrounding Saefftigen's pouch then extend posteriorly as cement gland ducts emptying into penis. Cement reservoir 1.1±0.1 (1-1.2) by 0.43±0.03 (0.40- 0.45). Saefftigen's pouch elongated 1.3±0.2, (1.2-1.5) by 0.70±0.06 (0.62-0.75). Bursal cap serrate in appearance, bursa well developed, muscular measuring 0.9-1 by 0.3-0.4 (Fig. 9). Male genital pore slit-like, terminal (Figs 21).

DISCUSSION

More new species of Acanthocephala are being described by researchers each year as well as previous descriptions being reviewed [4, 16]. This includes research on the genus *Sphaerirostris* [13]. A recent tool for descriptions of worms has been the electron microscope both SEM and TEM [17, 18]. The taxonomy of the genus *Sphaerirostris* has based mainly on hooks of the proboscis especially on the number and orientation of the armature rows Yamaguti, [1, 19, 20]. Amin [3] h as published lists of all the known species of Acanthocephala including the *Sphaerirostris*. As mentioned previously, the taxonomy of *Sphaerirostris* Golvan [5] has been questioned since Golvan [5] established the subgenus. Genus *Sphaerirostris* was erected by Golvan [5] as a subgenus of *Centrorhynchus* Luhe, 1911 and included 21 species with polydendritic lacunar system and having 3 or 4 tubular cement glands. Later, Golvan [6] listed 26 species by reversing the synonymies that he noted earlier Golvan [5] without any explanation. Amin *et al.* [21] listed 20 species valid of the genus *Sphaerirostris*. The present is comparatively similar to *S.picae* (Rudolphi, 1819) Golvan [5] but differs in proboscis armature which in the present specimens are16-20 rows of hooks with 8-10 hooks and 14 -16 rows of spines having 5-8 spines, the apical area of proboscis also armed. As compared to *S. picae* (Rudolphi, 1819) Golvan, [5] described by Amin *et al.* [21] in *Pica pica*and *Corvus* spp. from northern Iran the hooks rows are 32-38each having 8-10 hooks, while the spines rows are 27-36 with 2-5 spines. The posterior end of females is somewhat pointed in the present specimens as compared to *S. picae*. The eggs surface in *S. picae* are smooth but have a wavy surface in the present species as obvious by scanning electron microscopy. In addition, our specimens of *Sphaerirostris,* from Minia governorate varied morphometrically from *S. winderi* described from Pakistan by Khanand Heckmann, [7]. (Table 1) that characterized by a spinous, slender body. The proboscis with 10 to 12 longitudinal rows of 16 hooks each. There are 6 to 8 rows of spines, each row with 14 to 15 spines. The proboscis showing a small apical spineless area. Keeping in view the specific differences, include hook and spine number and rows shape and size of the worm and surface pattern of the eggs the present forms are proposed to be new sp. within the genus *Sphaerirostri*s. With new locality records.

Table 1: Comparative data(mm.)of both sexes of *Sphaerirostries sp.nov*.recorded in the present study and other previously described species.

	El-Minia, Egypt	Northern Iran	Pakistan
	The present study	Amin *et al*, 2010	Khan and Heckmann, 2015
Character	*S. Pica*	*S. Pica*	*S. winderi*
Trunk in female	15.76-17.24 by 2.33-3.54	5.12-18.87 by1.12-2.50	5.76-6.24 by 1.33-1.40
Trunk in male	13. 80-16.12 by2.5. -3	5.12-13.00by1.20-2.10	8.80-15.16 by1.16-2.04
Anterior proboscis,LxWin female	0. 33-0.40-by 0.23- 0.30	510-593 by 374-447	0.45-0.49 by 0.28-0.31.
posterior proboscis,LxWin female	0.20- 0.37by0.27- 0.40	177-333 by 364-437	- -
Anterior proboscis,LxWin male	0.267-0.333 by0.300-0.373	426-520 by 343-385	0.62-0.80 by 0.32-0.42;
Posterior proboscis,LxW in male	0.186-0.200by0.300-0.333	156-312 by 260-374	--
Hook rows &hooks per row	16-20&8-10	32-38&8-10	10 -12& 16
Spine rows &spines per row	14-16&5-8	27-36&2-5	6-8&14-15
Apical area of proboscis	Armed	Spineless	spineless
Proboscis receptacle,LxWin female	1.04-1.08by0.20-0.38	988-1,625 by 260-354	- -
Proboscis receptacle,LxWinmale	0.9-1.1 by0.2 - 0.4	975-1,525by 250-350	0.75-1.12 by 0.31-0.52
Cement glands,(no.)	3	4	4
Female posterior end	pointed	rounded	pointed
Gonopore	subventral	subventral	subterminal
Eggs,LxW	0.040-0.049 by0.013-0.018	45-60 (53) by 17-24	0.045-0.048 by 0.024-0.026.
Male genital opening.	Terminal &surrowndbylips	ventral with nospecialized features	--

Type host:Hooded Crow (*Corvuscoronecornix)*)
Site of infection: Intestine
Type locality:*Ashmunin* city south El-Miniagovernorate(Egypt)
No. of specimens: 8males and 5 females from six hosts
No. of host examined:3 6
Name:*Sphaerirostri*ssp.Nov.

CONCLUSION

The Acanthocephalon parasite recorded in the present study possessed all of the characteristics features of Family Centrorhynchidae and by comparison with the mostsimilar species to the present parasite,it was observed that it is similar in morphology to *Sphaerirostris picae* described by Amin *et al.* [21] and *Sphaerirostris winderi* describedby Khanand Heckmann, [7] but differs in proboscis armature include hookand spine Number. The present parasite shows apical area of proboscis also armed,while the compared species showing a small apical spineless area. The taxonomy of the genus *Sphaerirostris* has based mainly on hooks of the proboscis especially on the number and orientation of the armature rows, the present forms recovered from hooded Crowtfrom El -Minia province are proposed to be new sp. within the genus *Sphaerirostri*s. With new locality records.

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