ULTRASTRUCTURAL STUDY OF TWO PARASITES INFECTING DOMESTICATED TURKEY *MELEAGRIS GALLOPAVO* LINNAEUS, 1758 (GALLIFORMES: MELEAGRIDINAE) QENA, EGYPT

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Abstract

The work gave a detailed systematic morphology by optic and scan electron microscopy of two parasites; *Raillietina echinobothriida* Megnin, 1880 and *Spirora meleagaris* n. sp. infecting domesticated turkey, with some important description characters. SEM revealed that tegument of *R. echinobothriida* exhibits, filamentous, microtriches and sensory papillae densely covered the tegument of entire body, rostellum armed with two rows of hummer-shaped hooks and provide by 16-20 rows of small, rose thorn-shaped accessory spines. In addition, a number of taxonomic features in *S. meleagaris* n. sp. that differ from other species of the genus, mouth circular, bounded by a cuticular three circles plates, five pairs of cephalic papillae, an inner circle of two pairs situated on the wall of the buccal cavity, one pairs of larges sub-median amphids, and an outer circle of two pairs papillae. Buccal cavity supported by four chitinious cusped molar teeth anteriorly directed .Vulva near the end of the first third of the body, vulvular lips prominent. The male has unique rose like shaped pedunculated and unarranged numerous distributed sessile cervical papillae at the second third of the body that are distinguishable from other spirorid.

Key words: Egypt, Raillietina echinobothriida, spirorid parasites, Spirora meleagaris n. sp.

Introduction

The domestic turkey; Meleagris gallopavo Linnaeus, 1758 is a large bird, one of the two species of genus *Meleagris*, which meat ranks second in the poultry sector, and compares favorably with domestic chicken for meat and egg production. The increasing economic importance comes from low fat meat and eggs as major sources of protein and its feathers for decorative purposes. The increase in the zoonosis incidence more than 200 diseases were reported (Klimpek et al, 2007). Data on turkeys' helminthes and the pathogenesis are few. In Brazil, Barretto and Mies-Filho (1942) and Brener et al. (2006) described the pathogenicity of trematode; Paratanaisiabragai, nematodes; Cheilospirurahamulosa, Heterakis gallinarum and protozoan; Histomas meleagridis infecting domestic turkeys. In Egypt, Bould et al. (2009) stated that coccidiosis was a major intestinal parasites of poultry associated with severe economic losses and welfare issues. Barakat et al. (2012) reported a high seroprevalence in free range chickens (housereared) and incriminated poultry as source of zoonotic toxoplasmosis. Zoonotic helminthes lower the bird industrial productivity and may be fatal by gastrointestinal blocking (He et al, 1990). Bawe et al. (2005) reported lesions associated with gastrointestinal parasites in Nigerian fowls. Chicken cestodiasis not only cause body weight loss but also may cause many problems as enteritis, blood loss, production loss, nervous manifestations and death (Shahin et al, 2011). Adult tapeworm infects fowl causing stunted growth of young chicken, adult emaciation and decreased egg production (Samad et al, 1986). Brener et al. (2006) reported that lesions caused by the nematode Cheilospirura hamulosa Diesing, 1851 in Brazilian turkeys other as chickens and pheasants, caused severe pathogenicity. Rahman et al. (2009) showed that endo-parasites of scavenging chickens had a significant effect on weight loss. The economic importance of the poultry industry, as well as the fact that domestic fowls are kept by many private households to augment their income, necessitated a better understand of gastro-intestinal parasites, and prevention (Bussière et al, (2015).

Tapeworms (Davaineidae) included 14 genera of parasites, of which *Raillietina* was a major pathogenic for poultry (Schmidt, 1982) and caused loss in production (Kassai

et al, 1999). The tape-worms lack mouth and digestive system and thus all nutrients passed via body wall (Read et al, 1963). The tegument surface being covered with specialized microvilli, resembling the brush of mammalian enterocytes (Read, 1955) referred to as microtriches (Rothman, 1963), microvilli (Beguin, 1966) and tegumental projections (Morseth, 1966). Tegument had a high degree of morphological specialization to perform diverse functions as nutrient absorption, digestion, protection, excretion, anchoring and locomotion (Jones, 1975; Thompson et al, 1980; Coil, 1991; Hayunga, 1991; Palm et al, 1998).

Nematodes (Spirurida Oerley, 1885) infect vertebrates Alvareze *et al.* (1995) found that they parasitized submucosa and lived free in lumen of esophagus and stomach. Absence of host specificity and variations of specific characters made taxonomy of this group of nematodes very difficult. Genus Spirura Blanchard, 1849 included 36 species infecting vertebrate's worldwide (Smales, 2001).

The present study aimed to give more detailed morphological and taxonomical studies of the domestic turkey by light and electron microscopic examination.

Materials and methods

In this study eight birds locally bred domestic turkeys were sacrificed and the alimentary canals dissected out for helminthes parasites, intestinal mucosal was scrap and the contents was washed several times with physiological saline (0.9%) and after sedimentation, the sediments were examined under dissecting microscope. Only three turkeys were infected (48.4%). The recovered parasites were washed in normal saline solution, fixed in 10% buffered formalin, stained in Acetic-Carmine, dehydrated in ethanol and mounted in Canada balsam. Drawings were made by a camera Lucida.

For SEM studies, recovered samples were washed in phosphate buffered saline and fixed overnight in 2.5% gluteraldehyde (PH 7.4) at 4°C, washed three times in phosphate buffer and post fixed in 1% Osmium tetra-

oxide in 0.1% M phosphate buffer and dehydrated in ascending series of ethanol. Complete dehydration was performed in two changes of absolute ethanol. Specimens were mounted on stubs with double adhesive tape, coated with gold, and then examined with a high resolution scanning electron microscope (Joel 1200 Ex II).

Results

The identification of cestodes were based on Khalil's key (1994) and nematodes on keys of York and Mapleston (1926).

1- Raillietina echinobothriida Megnin, 1880 Fuhrmann, 1924 (Plates 1, 4): Order: Cyclophyllidea, Family: Davaineidae Braun, 1900, Subfamily: Davaineinae Braun, 1900

Adult *R. echinobothrida* has a characteristic structure composed of a series ribbon-like body segments, gradually enlarging anteriorly towards the posterior end, whitish in color, elongated, dorso-ventrally flattened, fresh ones measure 21-25cm with a mean (23cm). Body is wide, and rostellum armed with two rows of hooks which are hammershaped of 130-185μm in length, armed with16-20 rows of small, and rose-thorn-shaped accessory spines11-15 μm.

Scolex is globular, small, provided with strongly muscular four suckers, measuring 0.32-0.46mm long, by 0.37-0.53 mm. The neck is long and broad measures 1.3-2.5mm in length. Immature and mature segments are broader than long (Fig.4a, b, c) measures 0.90-1.2X1.25mm. Common genital pores are unilateral alternating and regularly located at the posterior third of the lateral wall of segment. Ovary is median, post-equatorial, bilobed, measuring 0.36-0.41x0.29-0.42mm. Ootype is situated between ovarian lobes, with a small seminal receptacle runs medially to give a saccular vagina which turns laterally to open in the female genital pore at the genital atrium. Uterus is sac like situated medially, with a large compact vitellaine gland mass in the segment mid-line. Numerous testes (29-34) are situated in two lateral fields on both sides, measuring 72-80x71-86 um. Vas differences leads into a swollen internal seminal vesicle that enters the pouch forming cirrus. Gravid segments are longer than broader, measuring 3.7-4.3x1.6-1.8mm. Uterus beaked up into egg capsules.

At higher magnification, the suckers' margin, center, its surrounding region and the rostellum were covered by filamentous microtriches. Within the circlet of hooks on the rostellum, the slender and filamentous microtriches were densely packed together, with a tendency for some spikes to coalesce or show transverse connections in some areas similar microtriches on the suckers. These microtriches were slender, filamentous not as long as in rostellum, but more densely packed together forming characteristic tufts inside the sucker (Figs. 4 d, f, g, h). The tegument was entirely covered with filamentous microtriches and around genital pore hair like shape (Figs.4 g, h). In addition, few sharply-ending electron-dense points were seen on the suckers' margins, their cavities and rest of scolex region. Gravid proglottides basal region interrupted by genital pores surrounded by numerous small papillae, lacked microtriches suggested that they are specifically atrophied during transformation from immature to mature to gravid stage.

2- Spirora meleagaris n. sp. (Pls. 2, 3, 5, 6, 7): Nematoda Rudolphi, 1808, Spiruroidea Railliet & Henry, 1915, Order: Spiruridea Diesing, 1861, Family: Spiruridae Oerley, 1885, Subfamily: Spirurinae Raillit, 1915

Genus: Spirura Blanchard, 1849: Medium sized more or less filiform worms, anterior end abruptly attenuated with rounded apex, posterior end rounded, body thick transversely striated, annular furrows with longitudinal folds ridges running along the body seen posterior to the excretory pore; the striations are wide and transverse and each possesses longitudinal ridges or folds, mouth elongated terminally, surrounded by chitinous prolongation of thickened vestibule, 0.04µ in both sexes. Lips inconspicuous or absent, Chitinous buccal cavity (Pseudobuccal capsule) or vestibule is present, with anterior end differentiated into two circular

pseudo labia; each pseudolabium with a single amphid and a pairs of oval cephalic papillae, a pairs of subcephalic papillae (Figs. 2a, c, 3c, 5f). The esophagus is long cylindrical and divided into two parts, a shorter anterior muscular portion and a longer glandular posterior one. Intestine is simple and without diverticula (Pl. 2a). Females are larger than males with prominent amphidelphic-vulvular lips, vulva with a welldeveloped sphincter located at body second third; vagina has muscular walls, giving rise to two uteri opposed, and containing immature eggs. Anterior ovary extends beyond junction of esophagus and intestine, but posterior one reaches anus, and eggs are smooth and unembryonated.

Female (Pls. 3, 5): Body, 12.8-21.3mm x 0.24-0.34mm. Transverse cuticular striae 2.5μ as highly corrugated towards posterior end giving the body a general rough appearance, muscular oesophagus 0.32-0.39mm x 0.088mm; glandular oesophagus 1.82x0.133 mm. Nerve ring 0.29-0.35mm and excretory pore circular 0.47-0.52mm from anterior end, vulva 3.9-7.8mm from anterior end; eggs 0.043x0.032mm, tail bounded in shape

Male (Pls. 2, 6, 7): Body 11-13x0.22-0.26 mm; transverse striae 0.9-2µ apart. Pharynx 0.09-0.14x0.019-0.023mm; muscular oesophagus 0.32x0.078mm; Nerve ring 0.28mm from anterior end, well small gubernaculum 0.042 x 0.062mm. Spicules are unequal dissimilar, longer spicule; 1.4-1.6x0.27-0.29 mm, with a ratio of 4:1between both. Caudal alae are well developed, supported by four preanal pairs of pedunculate papillae, Tail curved ventrally and bluntly. In both sexes, SEM showed that cervical region cuticle inflated to form a large cervical inflation, and also transversely, four large submedian cephalic papillae, four large of outer labial papillae and two lateral amphids lies on a circular-shaped borders on top of the cephalic plate, buccal cavity supported by four chitinious cusped molar teeth anteriorly directed and the worm cuticular surface is transversely annulated and each annulus is subdivided into 10-15 transverse striations; excretory pore small and circular surrounded by an elevated cuticular rim, cuticular transverse striae along entire body on posterior to excretory pore, with longitudinal folds. Striae width measures 1.5-2µm highly corrugated towards posterior end forming general rough body appearance. Female has a protruding vulva directed forwardly and elevated lip. Male has rose like shaped peduculated and unarranged numerous distributed sessile cervical papillae at body second third.

Discussion

Davaineidae cestodes was recovered from the intestine of a captive emu in Copenhagen (Krabbe, 1869), then Fuhrman (1909) described another species, from an emu in Berlin. The present material was well corresponded to description given by Movsesyan (2003) in poultry. Species of genus Raillietina are main parasites of poultry (Kassai, 1999). Morphological changes in the strobila tegumental surface involved erosion of folds in posterior region accompanied by an increasing degree of disorder in microtriches' arrangement. The microtriches' atrophy is due gravid segments voiding out from host body. Degeneration of microtriches enables above and at same time confirms its prime function as adhesion in host environment.

Ultrastructural study showed that proglottids are entirely covered with microvilli hairlike microtriches, which are the absorptive structures for feeding.

Smyth and McManus, 1989) reported that delicate microtriches serve as a surface amplifying structures, resemble functionally the intestinal mucosa of higher animals, and referred to amplified microtriches in the surface layer as brush-border. Rosario (1962); Rothman (1963) and Smyth (1969) added that this help worms in resisting intestinal peristaltic currents to maintain parasite's position. Blitz and Smyth (1973) reported that in *R. cesticillus* rostellar microtriches differed significantly from those on proglottides, by being longer and thinner. Electron dense cap was greatly reduced or absent.

Rothman et al. (1963) reported that absorption is limited to medullar base of microtriches with spike acting mainly for locomotion and attachment to favor an absorptive function for the rostellar microtriches. Thin microtriches mostly in sub-scolex region might represent increased sites of absorptive and secretory activity. Teguments in the scolex, immature and mature segments were lined by posteriorly directed filamentous microtriches, interspersed with few short blade-like microtriches. But, the tegument covering the pregravid and post gravid regions of strobila was apparent disintegration of the microtriches in small rounded tips. This agreed with Berger and Mettrick (1971) and Andersen (1975) who found that the gravid proglottides basal region was interrupted by genital pores surrounded by numerous papillae, without microtriches, as they are specifically atrophied during transformation of immature to mature and then to gravid stage. The degeneration of microtriches enables the above and at the same time confirms its prime functions adhesion in the host environment.

Diagnostic criteria of Spirura species were previously described from Africa and other parts worldwide. The present description of Spirurida differed significantly from that given by Lewis and Ashour (1983) and Jorge et al. (2012) in the number of cephalic papillae, presence of cervical papillae, and absence of pharyngeal teeth as well as in males presence of four precloacal and absent of two post cloacal papillae-like structures, and presence of longitudinal striae on caudal alae ventral surface. In the present specimens, there were pedunculated rose shaped papillae, and measurements of body length and width much reduced compared to sizes original given. Present species were closed related to the buff backed heron S. talpa (Mahdy and El-ghaysh, 1998) in measurement and caudal pedunculate papillae, almost four preanal pairs, and spicules unequal and dissimilar. The present S. meleagaris n. sp. has a number of distinguishing

taxonomic characters not reported from other species of the genus as mouth circular, bounded by a cuticular three circles of cephalic papillae, an inner circle of two pairs situated on the wall plate of buccal cavity, one pairs of large submedian amphids and an outer circle of two pairs papillae, buccal cavity supported by four chitinious cusped molar teeth anteriorly directed. Vulva lies near the end of first third of body and vulvular lips prominent. Male has unique rose like shaped peduculated unarranged numerous distributed sessile cervical papillae at second third of body distinguishing from other spirorid. The new species shared some characters with other spiruridae, but differed in a unique characters as body thick transversely striated, cuticle with rugae or as longitudinal folds and external raised incomplete annulations; branched and interrupted on cuticle surface helps creeping and migration within host, mouth circular, bounded by a cuticular three circles of cephalic papillae, an inner circle of two pairs situated on the wall of buccal cavity, one pairs of larges submedian amphids, an outer circle of two pairs papillae, and buccal cavity supported by four chitinious cusped molar teeth anteriorly directed. Amphids have assumed to be chemoreceptors because they open to outside, vulva near the end of body first third, vulvular lips prominent. Male has unique rose like shaped peduculated and unarranged numerous distributed sessile papillae at the third end of the body distinguishing from other spirorid. The chitinous 4 teeth are probably used during penetration into and migration through intestinal wall; while the different types of sensitive papillae and gabernaculum served for orientation during mating.

The present *S. meleagaris* n. sp. showed taxonomic features differ from other species, mouth circular, bounded by a cuticular three circles of cephalic papillae, an inner circle of two pairs situated on wall of buccal plate, one pairs of larges submedian amphids, and an outer circle of two pairs papillae, buccal cavity supported by four chitinious cusped

molar teeth anteriorly directed. Male has unique rose like shaped peduculated and unarranged numerous distributed cervical sessile papillae at the second third of body.

SEM showed a number of characters differ from *S. dentate* (Alvareze *et al*, 1995) in four simple denticles on oral cavity inner; a pairs of longitudinal ridges along body, a second pair of papillae tail in second third, denticles four molar cusped teeth and longitudinal folds with caudal papillae.

Conclusion

The present material have characters of Spirura Blanchard,1849 and included as a new species as mouth have two circles of cephalic papillae, an inner circle of four larges papillae situated on wall plate of buccal cavity just inside mouth and an outer circle of four large submedian papillae and two lateral amphids, buccal cavity supported by four chitinious cusped molar teeth anteriorly directed, several types of cephalic, cervical and caudal sensory papillae are present and female vulva near to the middle of the body.

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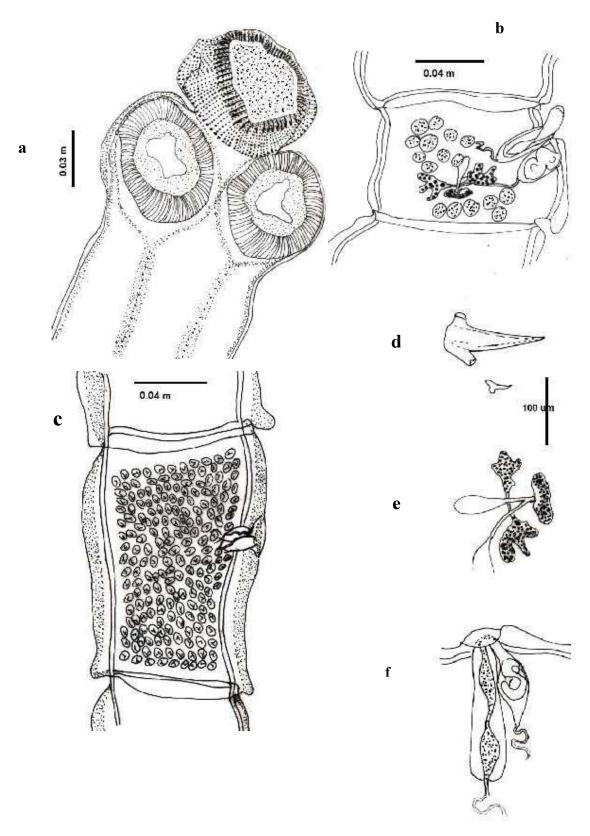


Plate 1: Camera lucida drawing *R.echinobothriida* .(a) scolex with long neck showing rostellum with 2 rows of hammer shaped hooks, (b) mature segment have numerous of testes(C) gravid proglottide (d) hooklets(e) a pair of lobed ovaries(f) genital opening and cirrus pouch.

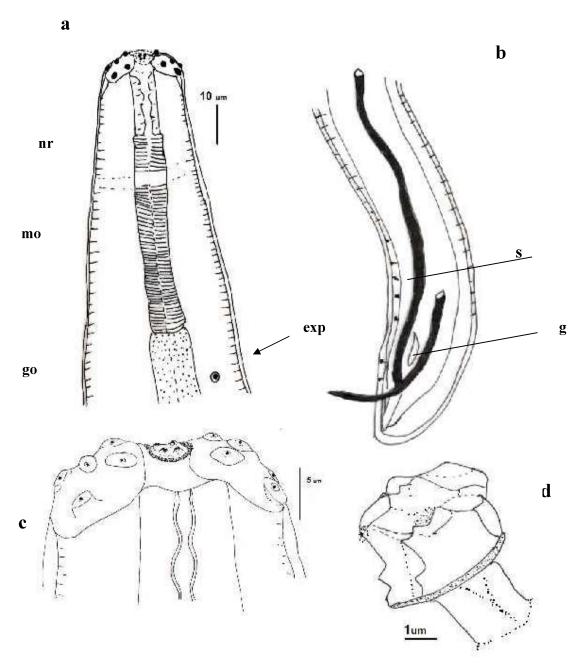


Plate 2: Camera Lucida drawing male S*pirora meleagaris* n. sp. of anterior and posterior ends showing arrangement of cephalic papillae and teeth, (a,c), gubernaculum (g), unequal spicules (b), pedunculated rose shape papilla(d).

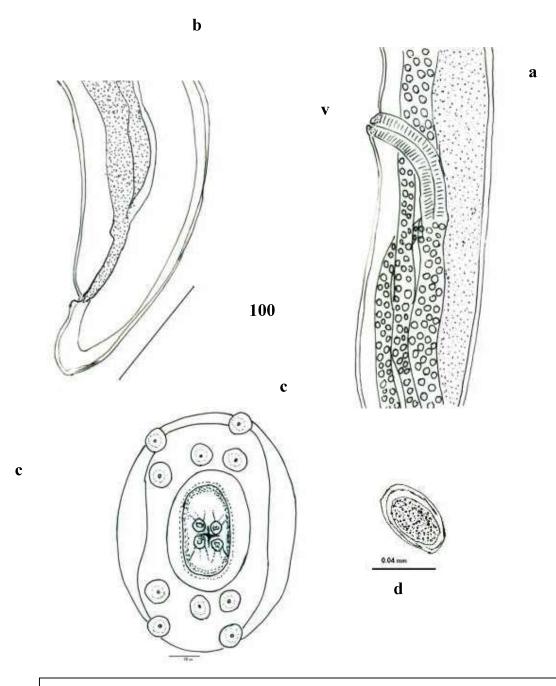


Plate 3: Camera Lucida drawing females of *Spirora meleagaris* n. sp. (a) Vulva region, (b) posterior end, (c) oral aperture of en faces apical view. (d) Egg.

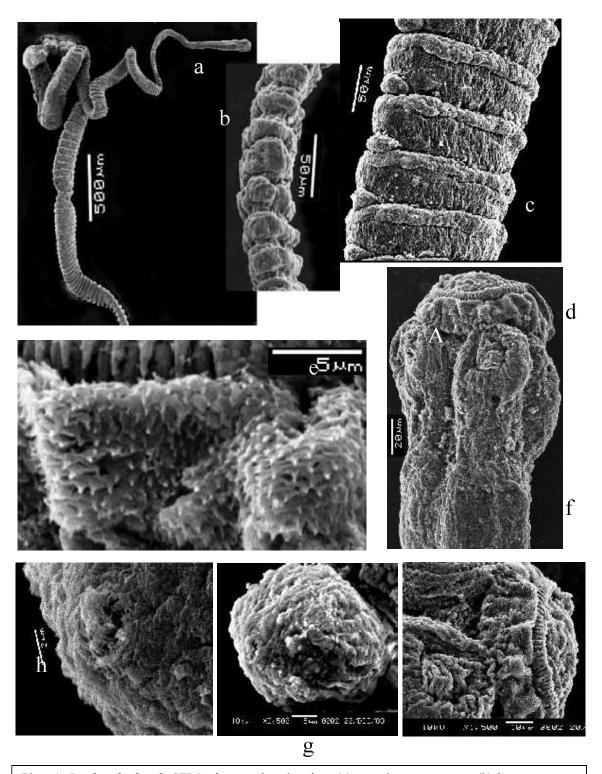


Plate 4: *R.echinobothriida* SEM micrographs showing; (a) complete tapeworm, (b) immature segments, (c) mature segments showing cirrus in genital opening., (d) scolex with long neck illustrating it bears four suckers and evaginated rostellum (e&f) high magnification of rostellum showing crown hooks and rows of hooklets and tegumental surface of the sucker region showing uniform filamentous microtriches densely packed together tufts inside the sucker forming characteristic., (g&h) high magnification of longer cirrus poush showing filamentous microtriches

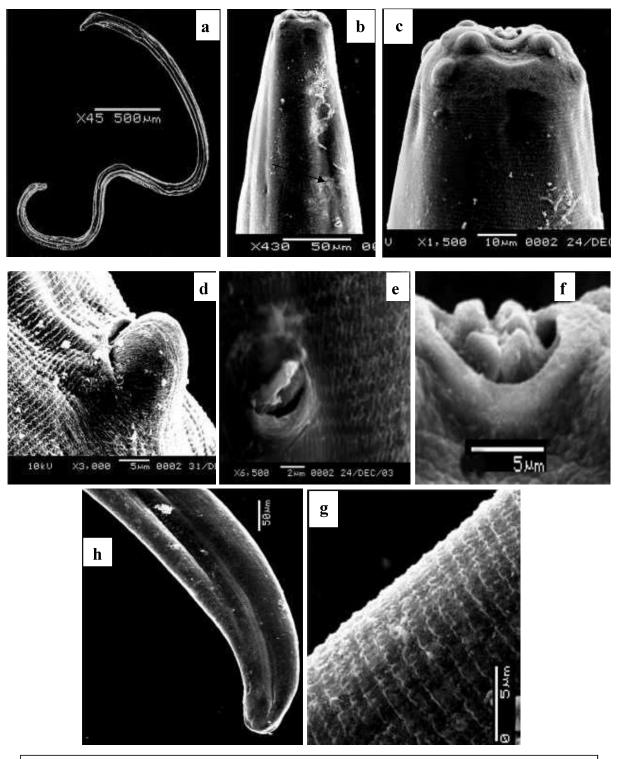


Plate 5: SEM micrographs *Spirora meleagaris* n. sp. female showing whole mount (a), anterior end with excretory pore(b), high magnification of mouth showing arrangement of cephalic papillae and 4 molar teeth (c),vulva (d).excretory pore (e), 4 molar cusps teeth (f), cuticle transverse striations (g),posterior end(h).

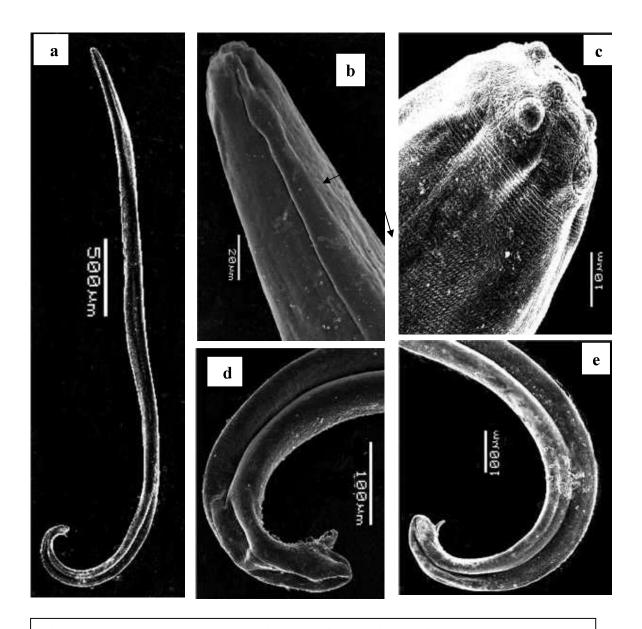


Plate 6: *Spirora meleagaris* n. sp. male SEM micrographs showing whole mount (a), anterior end showing beginning longitudinal folds and excretory pore (b,c),high magnification of the mouth note beginning of transverse striation, (d, e) posterior end showing unequal spicules with caudal papillae, longitudinal folds and tail blunt, with small caudal alae and slightly curved ventrally.

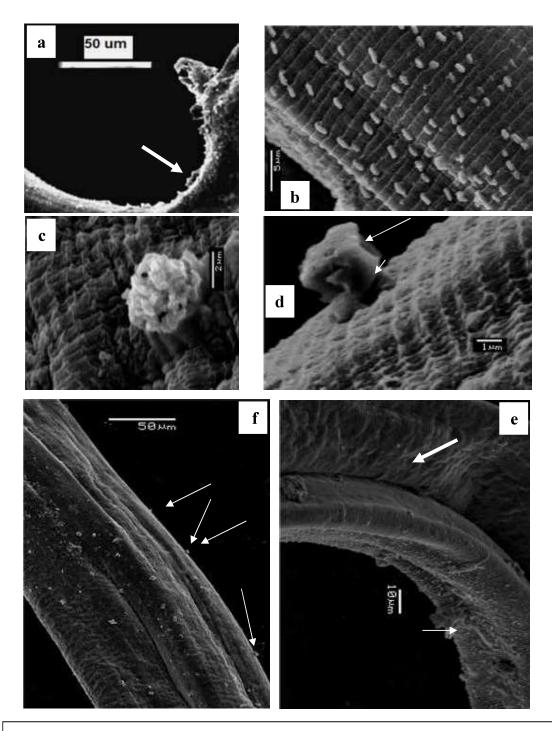


Plate 7: Spirora meleagaris n. sp. male SEM micrographs showing high magnification of the posterior end with caudal papillae (a), numerous unarrangement sessile Cervical papilla (b), explosive or impaired gland (c), unexploded or booming gland (pedunculated rose shape papilla Cervical papilla) (d), cervical region and cervical papilla, longitudinal folds (e), male cervical region; cuticular surface with pedunculated rose shape papilla, transverse striations(f).