

RESEARCH ARTICLE

**SCANNING ELECTRON MICROSCOPY OF *MICROTETRAMERES SPIRALIS* (NEMATODA, FAMILY TETRAMERIDAE) PARASITISING CATTLE EGRET “*ARDEOLA IBIS IBIS*” IN EGYPT**

**Hewaydah E. Abou Shafeey\***

Department of Zoology, Faculty of Science, Tanta University, Gharbia, Egypt

**ABSTRACT**

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**\*Correspondence:**

Hewaydah Abou Shafeey  
Zoology Department  
Faculty of Science  
Tanta University  
Gharbia, Egypt  
E-mail:  
[hewaydah@yahoo.com](mailto:hewaydah@yahoo.com)

*Microtetrameres spiralis* is one of the common spirurid parasites recovered from the cattle egret “*Ardeola ibis ibis*”. No data was available on its ultrastructural characteristics with scanning electron microscopy. Therefore, the present study aimed to investigate, in details, the characteristic features of *M. spiralis* in cattle egret using both the light and scanning electron microscopies. The results revealed that nine out of seventeen *Ardeola ibis ibis* (53%) were infected with *M. spiralis*. Female worms were deeply embedded in proventriculus wall; their bodies were twisted in longitudinal tight spiral forming 2-3 turns. Males move in lumen; sometimes they were lodged on lining. They have filiform cylindrical shape. Male possesses two unequal and dissimilar spicules. The buccal capsule is well-chitinized, thick-walled, flask-shaped in females, but tubular in males. The vulva lies at the posterior third of the body. Cuticle was sharp transverse annulated along whole length of the body without alae or spines. Each pseudolabium has inner and outer edges; the outer edge has wavy-shaped, triple swollen processes. The depressed part that located between the two edges of each pseudolabium contained three corrugated, flower-shaped processes. The anterior annulations are ridged and have numerous pointed triangular processes. A single raised corrugated papilla appeared. Button-like structures are distributed on longitudinal striations. An oval striated, papilla-shaped structure is elevated with coiled, rod-shaped projection. The transverse annulations over the mid-portion of the female’s body have wavy margins.

**INTRODUCTION**

Egypt is perfect transit for migratory birds, due to its miscellaneous habitats and temperate weather. Cattle egret, buff-backed heron “*Ardeola ibis ibis*”, is a cosmopolitan wild bird species of family Ardeidae that lives in agricultural lands<sup>[1]</sup>. The cattle egret is popular for its role in the biocontrol of cattle parasites, land pests, agricultural

insects, mollusks, and earthworms. Wild birds constitute a possible risk for domestic species and human being due to spreading of parasitic infections<sup>[2]</sup>. These birds feed on arthropods, mollusks, fishes, reptiles, and rodents, which many of them act as intermediate host for helminths<sup>[3]</sup>. In natural infection, the wild birds are found associated with the severe damage of gizzard and

proventriculus<sup>[4]</sup>. Heavy infestation with these parasites may result in death of the host<sup>[5]</sup>. In addition, these parasites caused great economic losses through decreasing feed efficiency and weight gain, as well as delaying sexual maturity and egg production<sup>[6]</sup>. In Egypt, different helminthic parasites of cattle egrets were studied<sup>[7-14]</sup>.

The family Tetrameridae (Nematoda) is characterized by the sexual dimorphism of the species. The body shape pattern of the female is the main characteristic key used to differentiate between genera of the family<sup>[15]</sup>. The most commonly reported parasites of herons (egrets) are tetramerid spirurid nematodes. Both subgenera *Tetrameres* and *Microtetrameres* were raised to generic category based on female body form; globular or spindle-shaped in *Tetrameres* and with its longitudinal axis spirally coiled in *Microtetrameres*<sup>[15]</sup>. The genus *Microtetrameres* currently includes several species parasitic in birds worldwide<sup>[1,3,16-19]</sup>. In the agricultural country like Egypt; man is usually in close interaction with water, therefore there is a potential danger of invasion with nematodes of birds. *Microtetrameres spiralis* is one of the common tetramerid nematode parasites that were previously reported from the Egyptian *Ardeola ibis ibis*; but inadequately described (with no morphological data for the females)<sup>[1,20]</sup>. The morphological description of the microtetramerid nematodes by scanning electron microscope (SEM) is lacking. Therefore, the present study was conducted to investigate, in details, the characteristic features of *M. spiralis* in comparison with previous reports using both light microscope and SEM.

## MATERIAL AND METHODS

### Collection of specimens

The proventriculus of seventeen *Ardeola ibis ibis* collected from Gharbia Governorate was examined for parasitic infection by the light microscopy. Nematodes were collected by light pressure on the proventriculus wall around the swollen lesions for collecting the female specimens. Male specimens were

collected from the mucus of the proventricular lumen and from beneath the lining of the proventriculus.

### Light microscopy

The collected specimens washed in normal saline 0.7%. Female and male specimens fixed in 70% ethanol, cleared in lactophenol and mounted in glycerine jelly. Mounted specimens were photographed using an Olympus C X 31 microscope and an Olympus imaging corp digital camera E-330 DC 7.4 V (Olympus MEA FZ-LLC, Dubai, UAE). The morphometric studies were based on randomly selected 40 specimens (20 males and 20 females) and measured by 0.01 mm microscope micrometre glass scale (PZO, Warszawa, Poland) and a calibrated lens.

### Scanning electron microscopy

Specimens were fixed in 3% phosphate buffered glutaraldehyde, post fixed in 1% osmium tetroxide, critical point-dried using the EMS 850 Critical Point Dryer (Electron Microscopy Sciences, Hatfield, PA, USA). Specimens were gold-coated (nearly 50 nm thickness) using an SPI Module<sup>TM</sup> Sputter Coater System (West Chester, PA, USA), and then examined and photographed using the high-vacuum mode of a JEOL JSM-5500LV SEM (Tokyo, Japan) at an accelerating voltage of 25 KV. SEM examination was based on 12 specimens, 4 males and 8 females.

## RESULTS

### Rate of natural infection

The examination of 17 birds (10 females and 7 males) revealed that nine (53%) were naturally-infected with tetramerid nematodes *M. spiralis* inhabiting the proventriculus, which also contained parts of insects and frogs among other food debris (Figure 1a). The nine infected birds were five females (29%) and four males (24%).

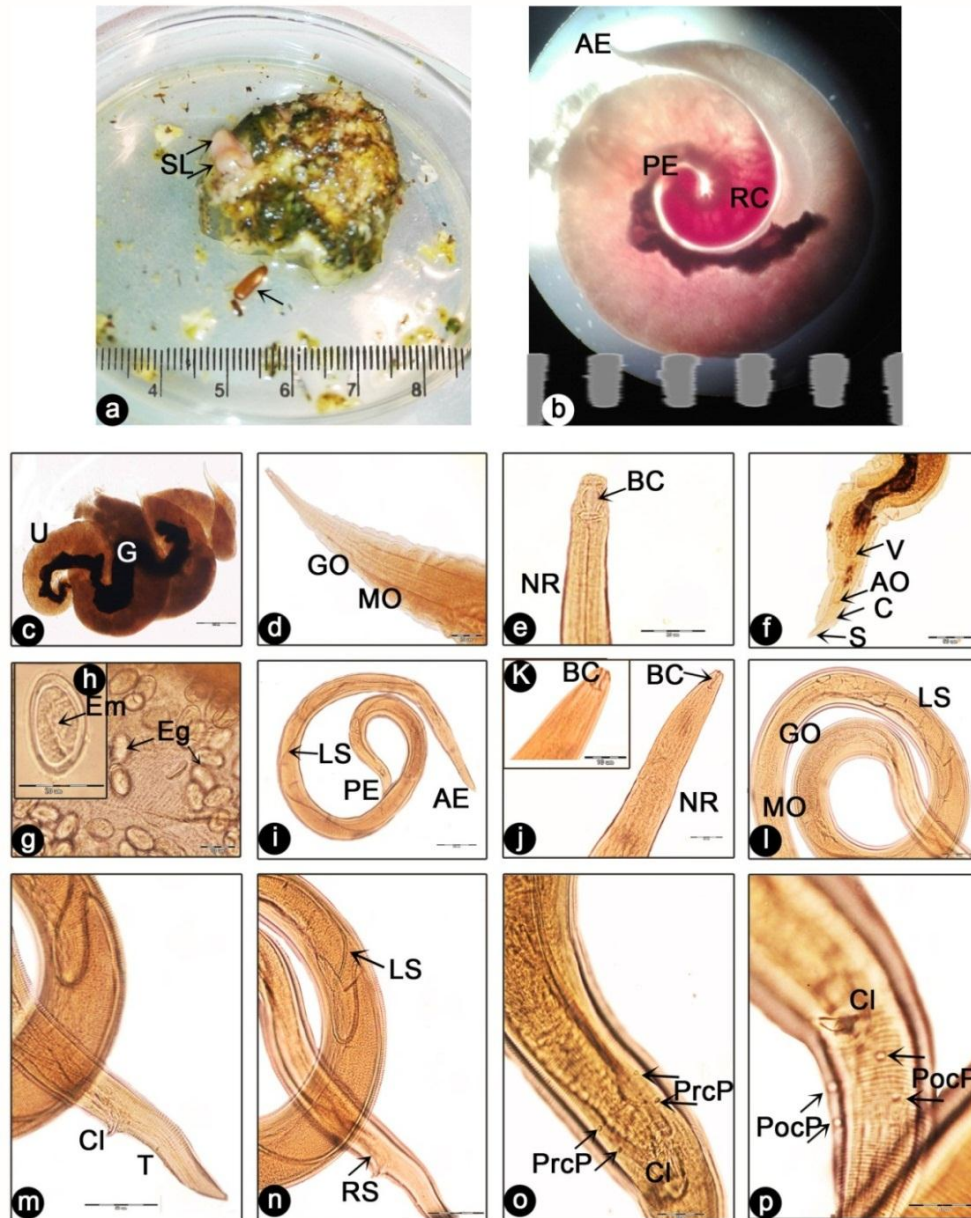
### Parasite description by light and scanning electron microscopy

The light microscopic examination of proventriculus revealed that the females of

*M. spiralis* were found deeply embedded in proventriculus wall (within the gastric glands) with suspended tails in the lumen of the proventriculus forming numerous swollen lesions (Figure 1a). The collected live adult females were bright red in colour and their bodies were twisted in longitudinal tight spiral, forming of two to three turns. The anterior and posterior ends protruded from the central portion and wrapped around body. Both ends are gradually pointed (Figures 1b and c). The female measured 3.3-3.8 mm in length, while the swollen globular mass of mid-portion of the body measured 0.863-1.099 mm in the diameter (Table 1). The anterior end has a small mouth that leads into a small, chitinous, thick-walled, and flask-shaped buccal capsule (Figures 1d and e), which measured 0.023-0.029 mm in length and 0.019-0.021 mm in width (Table 1). Gut is tubular with oesophagus composed of muscular part and glandular part. The muscular part of oesophagus (Figure 1d) measured 0.238-0.283 mm in length and 0.029-0.035 mm in width (Table 1). Meanwhile, the glandular oesophagus could not be measured correctly due to the corrugation of the specimens. The intestine is usually filled with detritus and ends in a narrow tube opening at the anus. The intestine appeared brown or black in colour (Figures 1b and c). Anal opening lies just posterior to the vulva at the distance of 0.204-0.238 mm from posterior tip of the tail (Figure 1f). The gravid females contain coiled uterus filled with enormous embryonated mature eggs. The eggs measured 0.036-0.051 mm in length and 0.017-0.032 mm in width (Table 1). Eggs are transparent, thick shelled and wide elongated with rounded ends, while their sides have one more flattened than other. The eggs contained fully developed embryo, which appeared as banana-like structure without seeming any movement (Figures 1g and h). The vulva lies a short distance in front of anus at 0.190-0.314 mm anterior to the anal opening (Table 1). The posterior extremity showed gradually elongated sharp end, with

a long pointed rod-shaped structure that may be withdrawn within the tail cuticle (Figure 1f).

Scanning electron micrographs of female *M. spiralis* showed that the buccal opening of the female appeared hexagonal in shape and surrounded with two pseudolabia with inner and outer edges; in between the two edges of each pseudolabium a depressed part was located. The outer edge has triple swollen (wavy-shaped) processes; and it is more elevated than the inner one, which also has triple wavy-shaped, but less swollen. The depressed part that located between the two edges of each pseudolabium contained three corrugated flower-shaped, regularly-distributed processes (Figure 2a). Each pseudolabium bears a single amphid and a pair of rounded pedunculated cephalic papillae (Figure 2a). Body of each papilla appeared longitudinally striated sides with a smooth cover (Figure 2b). The scanning electron micrographs illustrated that the cuticle was sharp transverse annulated along the whole length of the body without alae or cuticular spines. The cuticle over the globular mid-portion of the body was most deeply transverse annulated with wavy margins (Figure 2c). In the anterior part, the transverse annulations gradually appear slightly ridged and show numerous irregularly distributed, short pointed, triangular processes. A single raised corrugated papilla was obviously appeared (Figure 2d). The regular, parallel, longitudinally striations were obviously detected anteriorly in between the cuticular transverse striations. At higher magnification, small bulges raised button-like structures are irregularly distributed on the longitudinal striations in between transverse ones. An oval striated, papilla-shaped structure is elevated in between these longitudinal striations with small coiled rod-shaped projection in its centre (Figure 2e). Female posterior end is curved ventrally and showed numerous wide transverse pleats that lead to short flat collar-like part. The collar-like part leads to another long pointed part that has a tiny pointed tip (Figure 2f).



**Figure 1:** Photomicrographs of the proventriculus of *Ardeola ibis ibis* (a), female (b-g) and male (i-p) of *Microtetrameres spiralis*. (a) Enlarged part of the proventriculus showing part of insect (arrow), swollen lesions (SL). (b) Apical view of female specimen showing red colour (RC), anterior end (AE), and posterior end (PE). (c) Lateral view of spirally coiled female specimen (three body turns) showing the uterus (U) and gut (G). (d and e) High magnification of anterior part of female specimen showing glandular oesophagus (GO), muscular oesophagus (MO), buccal capsule (BC), and nerve ring (NR). (f) Posterior extremity of female specimen showing pointed structure (S), tail cuticle (C), vulva (V), anal opening (AO). (g) Enlarged part of the uterus showing eggs (Eg). (h) High magnification of an egg contained fully developed embryo (Em). (i) Male specimen, left spicule (LS). (j-l) High magnification of the anterior end of male specimen. (m) Caudal end of the male specimen, tail region (T), cloaca (Cl). (n) Venterolateral view of posterior extremity of the male specimen showing flexed left spicule (LS) and right spicule (RS). (o) Ventral view of enlarged caudal part showing precloacal papillae (PrcP). (p) Ventral view of enlarged caudal part showing postcloacal papillae (PocP). Scale bar: 10 µm (g and k), 20 µm (d, e, h, j, o, and p), 50 µm (f and l-n), 100 µm (c and i), 1000 µm (b).

**Table 1:** Comparative measurements (mm) of females *Microtetrameres spiralis* detected in Egypt.

	<i>Microtetrameres spiralis</i> (Mahdy and El-Ghaysh <sup>[1]</sup> )	<i>Microtetrameres spiralis</i> (current study)
Host	<i>Ardeola ibis ibis</i>	<i>Ardeola ibis ibis</i>
Locality	Abo-Rawash, Giza province	Tanta, Gharbia province
Body length	3.1-3.6	3.3-3.8
Body width	ND	0.863-1.099
Buccal capsule length	0.021-0.03	0.023-0.029
Muscular oesophagus length	ND	0.238-0.283
Oesophagus width	ND	0.029- 0.035
Eggs length	0.033	0.036-0.051
Eggs width	0.016	0.017-0.032
Vulva	Anterior third of the body	Posteriorly (distance to anus 0.190-0.314)
Tail length	0.27-0.34	0.204-0.238

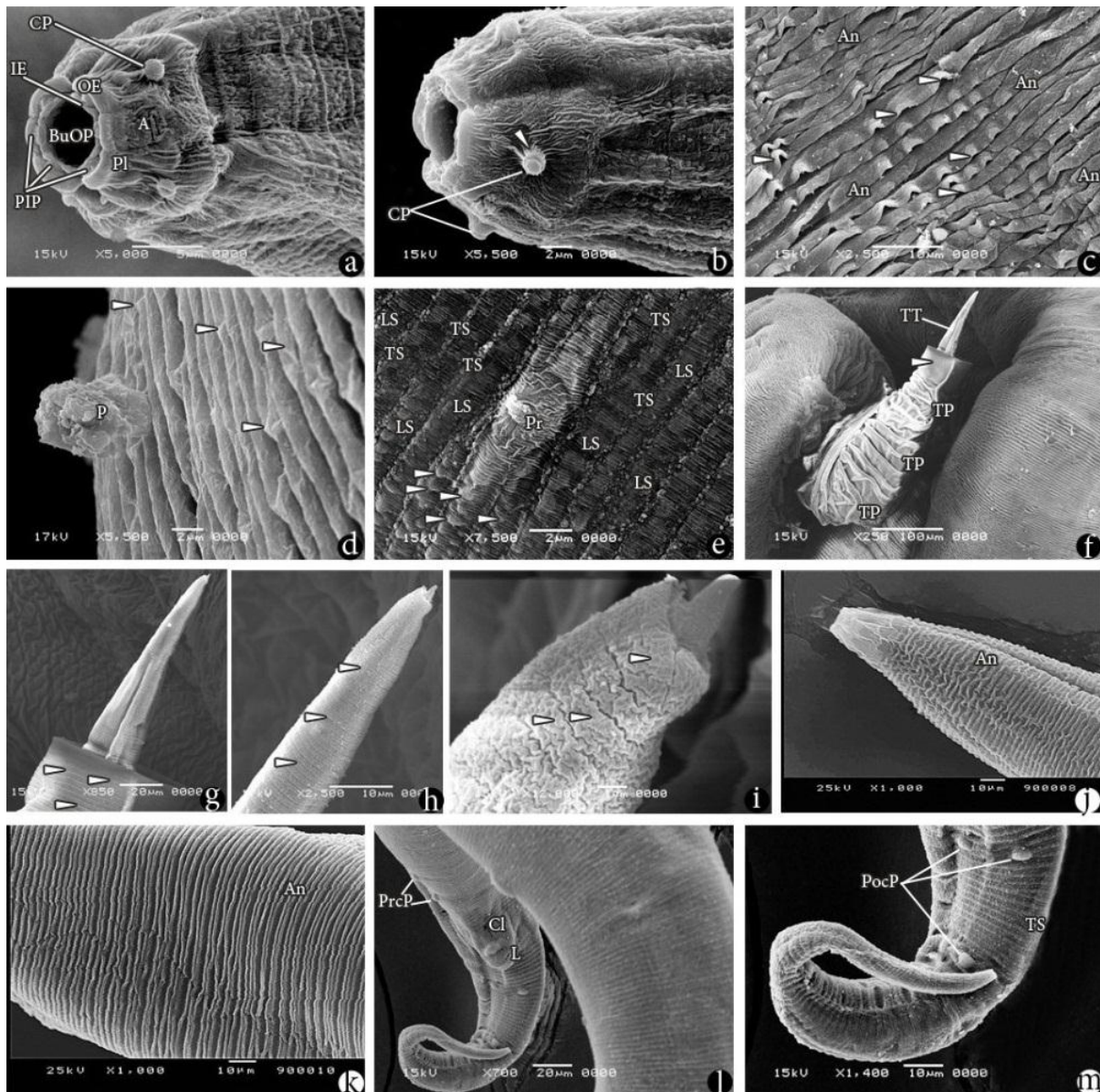
ND: not determined

The cuticle over the posterior end showed finely delicate cuticular transverse striations (Figures 2g and h) of collar-like part from which projected the caudal conical extremity. The cuticle of the posterior tip was inflated, where the higher magnification showed the cuticular transverse striations are gradually merged into irregular corrugations (Figure 2i).

The light microscopic examination revealed that the males of *M. spiralis* apparently moved freely in the lumen of the proventriculus; sometimes few males were lodged on the lining or occurred in the mucus of the proventricular lumen. Males were medium-sized, whitish in colour, and filiform, cylindrical in shape. Their body was stout and curled ventrally towards the posterior end (Figure 1i). Male body length was 3.3-4.2 mm, and the width at about their middle part was 0.11-0.14 mm (Table 2). The mouth leads into a small, well chitinized, thick-walled, and tubular buccal capsule; 0.19-0.29 mm in length (Table 2) and 0.009-0.008 mm in diameter (Figures 1j and k). The oesophagus was clearly divided into two regions, an anterior muscular and a posterior glandular parts (Figures 1j and l). The

length of the muscular part of oesophagus measured 0.268-0.393 mm, while its width measured 0.032-0.038 mm at the middle part of the body (Table 2). It is encircled by the nerve ring about 0.133-0.146 mm from the anterior end (Table 2). The length of the glandular oesophagus measured 0.353-0.471 mm, and its width measured 0.046-0.058 mm (Table 2). The caudal end of the male worms is ventrally curved. The tail is elongated, non-alate, and conical in shape; it measured 0.228-0.247 mm in length (Table 2), and gradually pointed extremity with rounded tip (Figures 1i and m). Each male possesses two spicules, very unequal in length and dissimilar in shape. The left spicule is well chitinized and slender; its proximal and distal terminations are slightly rounded. It is remarkably long, extending anteriorly, and when not extruded it flexed and recur internally near the male mid-body (Figures 1l and n). Its proximal termination is situated just behind the end of the oesophagus and measuring 2.292-2.748 mm in length (Table 2). The right spicule is less heavily chitinized, short arcuate, and not easily obvious (Figure 1n); it measured 0.139-0.173 mm in length (Table 2).





**Figure 2:** Scanning electron micrographs of female (a-i) and male (j-m) of *Microtetrameres spiralis*. (a) Subapical view of the anterior end of female specimen showing the buccal opening (BuOp), two pseudolabia (Pl), outer edge (OE), inner edge (IE), pseudolabia processes (PIP), cephalic papillae (CP), and an amphid (A). (b) Lateral view of the anterior end showing the longitudinally striated stalk (arrowhead) of the cephalic papillae (CP). (c) Female mid-body cuticle showing transverse annulations (An) and wavy margins (arrowheads). (d) High magnification of annulations showing triangular processes (arrowheads) and corrugated papilla (P). (e) Cuticular transverse striations (TS) of the anterior part of the body, longitudinally striations (LS), projection (Pr), and bulges (arrowheads). (f) Posterior extremity showing curved tail with transverse pleats (TP), collar-like part (arrowhead), tail tip (TT). (g and h) Enlarged posterior extremity of collar-like part showing cuticular transverse striations (arrowheads). (i) High magnification of female tail posterior tip showing cuticular corrugations (arrowheads). (j) Anterior part of male specimen showing cuticular transverse annulations (An). (k) Enlarged cuticular transverse annulations (An) near middle male body part. (l) Posterior part of male showing cloaca (Cl), lips (L), and preloacal papillae (PrcP). (m) Higher magnification of male posterior part showing delicate transverse striations (TS), towards the posterior tip, and postcloacal papillae (PocP).

**Table 2:** Comparative measurements (mm) of males *Microtetrameres spiralis* detected in Egypt.

	<i>Microtetrameres spiralis</i> (Mahdy and El-Ghaysh <sup>[1]</sup> )	<i>Microtetrameres spiralis</i> (current study)
Host	<i>Ardeola ibis ibis</i>	<i>Ardeola ibis ibis</i>
Locality	Abo-Rawash, Giza province	Tanta, Gharbia province
Body length	4.0-4.2	3.3-4.2
Body width	ND	0.11-0.14
Buccal capsule length	0.17-0.21	0.19-0.29
Muscular oesophagus length	ND	0.268-0.393
Muscular oesophagus width	ND	0.032- 0.038
Glandular oesophagus length	ND	0.353-0.471
Glandular oesophagus width	ND	0.046- 0.058
Nerve ring	ND	0.133-0.146
Left spicule length	2.2-2.5 (simple tip)	2.292-2.748
Right spicule length	0.13-0.15 (point tip)	0.139-0.173
Left/right spicule ratio	16:1	16:1
Tail length	0.23-0.28	0.228-0.247
No. of precloacal papillae	3	2 pairs
No. of postcloacal papillae	3	2 pairs

ND: not determined, No.: number

The left/right spicule ratio is 16:1. Each male possesses four pairs of sessile genital papillae near the cloacal aperture, two pairs precloacal and two pairs postcloacal; ventrally bilaterally symmetrical (Figure 1p).

Scanning electron micrographs of male *M. spiralis* showed that the deeply cuticular transverse annulations appeared along the length of the body (Figures 2j and k). The cloaca has protuberant, elevated lips with elongated aperture; the cuticle of lips is irregularly corrugated (Figure 2l). Precloacal and postcloacal papillae appeared sessile, oval in shape. The cuticular annulations appeared as delicate striations towards the posterior tip (Figure 2m).

## DISCUSSION

The present study elucidated one tetramerid worm that invaded the buff-backed heron (*Ardeola ibis ibis*). The described nematode was assigned to the genus *Microtetrameres* depending on the body of the female (twisted in tight) and male (without spines on lateral sides). The identification based on

this criterion agrees with other studies that adopted and considered *Microtetrameres* as a separate genus<sup>[15,21-25]</sup>. The presence of some parts of insects' bodies within the proventricular cavity of the buff-backed heron in the current and previous studies<sup>[3]</sup> proved that different insect species played a role in the transmission of *M. spiralis* and other nematodes to the local aquatic birds. In the present study, the prevalence of *M. spiralis* collected from buff-backed heron (*Ardeola ibis ibis*) of Gharbia province was higher than that was previously recorded (20-40%) by other studies<sup>[1,20]</sup>. These variations may probably due to the locality and distribution of the intermediate host.

The present data detected that *M. spiralis* microhabitat is the proventriculus of buff-backed heron. The females are typically embedded in the gastric glands, permanently occupied the crypts of Lieberkühn, with their tails directed towards the lumen of the proventriculus. However, males are generally found on the mucosa or in the

lumen of the proventriculus moving freely between the crypts and the lumen. This finding confirms the previous suggestion that the males move nearby spontaneously to reach and fertilize the immobile females<sup>[18,26]</sup>. The living females of *M. spiralis* that were collected from the epithelial lining of the proventricular glands in adjacent connection with their anterior ends have a red colour. The red colour may be due to the haemoglobin of the host's blood as reported in *Tetrameres confuse*<sup>[22]</sup>. This finding is in agreement with the previous suggestion of Clark *et al.*<sup>[17]</sup>. They suggested that the presence of avian nucleated red blood cells within the alimentary canal of female *Microtetrajueres sturnellae* and *Microtetrameres nestoris* indicates that these parasites are haematophagous. In addition, Clark *et al.*<sup>[17]</sup> reported that the presence of the parasite caused the destruction of glandular secretory cells of the proventriculus leading to loss of their secretory function.

The morphometric analysis of the obtained *M. spiralis* closely coincided with the specific characteristics described by Mahdy and El-Ghaysh<sup>[1]</sup> except for few measurements (Tables 1 and 2). These differences may possibly be due to the different geographical locality. The length, width, and other dimensions of the studied specimens including the spicules ratio of *M. spiralis* male were within the ranges mentioned by Mahdy and El-Ghaysh<sup>[1]</sup>. However, the males have longer buccal capsule and the tail length is much greater than that defined by Mahdy and El-Ghaysh<sup>[1]</sup>. In the present study, precloacal and post cloacal papillae are two pairs, while in the study of Mahdy and El-Ghaysh<sup>[1]</sup> the precloacal and post cloacal papillae are three pairs. The body length of *M. spiralis* females and the buccal capsule length in the current study are closely similar to the previous finding of Mahdy and El-Ghaysh<sup>[1]</sup>. The vulva located at the posterior third of the body in the current work; whereas in other study, it located at the anterior third of the body<sup>[1]</sup>.

Unfortunately, there are no available SEM descriptions to *Microtetrameres* sp. for comparing with the current results. Only little information is available concerning details of SEM study for genus *Tetrameres*. The present data detected that the buccal opening of *M. spiralis* is surrounded by two developed pseudolabia. This was correlated with Quentin *et al.*<sup>[19]</sup> suggestion who reported that the sub-genus *Microtetrameres* has a primitive nature of its family; where Tetrameridae represented by the presence of developed pseudolabia, which resemble the cephalic structures in the Spiruridae. In Egypt, the morphological description of *M. spiralis* particularly by scanning electron microscopy is lacking. In the current study, SEM description of *M. spiralis* revealed three corrugated flower-shaped processes deeply located between the two edges of each pseudolabium. The cuticular annulations have wavy margins anteriorly with numerous pointed triangular processes. Scanning electron micrographs also illustrate that the cuticle is transversely annulated with longitudinal striations near the anterior extremity of the females. In addition, numerous regularly arranged buttons-like bulges were sited. These detected structures may be have a function in obtaining blood as a food from the proventriculus glandular epithelium of the host as previously suggested<sup>[27]</sup>. The ultrastructure at the oral opening of *Tetrameres mohtedai* female was interpreted as a significant structure that induced pathology in terms of laceration of the glandular epithelium and the tissue during blood feeding from the host<sup>[27]</sup>. In addition, the scanning electron micrographs of the current study revealed a single papilliform structure with central coiled projection. This structure resembled the papilliform structures at various regions of the body length of *Tetrameres (Gynaecophila) aspicula*, which combined tactile and secretory functions<sup>[24]</sup>. Also, it looked like the shape of the somatic papilla of the *Tetrameres (Gynaecophila) spirospiculum*<sup>[28]</sup>.



## CONCLUSION

The SEM, in the current study, cleared some important characteristic features of *M. spiralis* including surface topography of adult female, the flower-shaped processes located between edges of each pseudolabium, the numerous pointed triangular processes in the anterior ridged cuticle, and the button-like structures that are distributed on the longitudinal striations. Further studies, by X-ray analyses, are in progress to reveal the chemical nature of the papilla-shaped structure.

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استخدام الميكروسكوب الإلكتروني الماسح في دراسة طفيلي "*Microtetrameres spiralis*"  
من الديدان الاسطوانية فصيلة "Tetrameridae" المتطفل على  
طائر أبو قردان "*ARDEOLA IBIS IBIS*" في مصر

هوايدة السيد أبو شافعي

قسم علم الحيوان، كلية العلوم، جامعة طنطا، الغربية، جمهورية مصر العربية

يعتبر الطفيلي "*Microtetrameres spiralis*" من الطفيليات الشائعة التي تصيب طائر أبو قردان. ولا يوجد دراسات سابقة تُظهر التركيب الدقيق لهذا الطفيلي بواسطة المجهر الإلكتروني الماسح. ولهذا هدفت الدراسة الحالية إلى وصف الخصائص التركيبية المميزة لهذا الطفيلي باستخدام تقنيات المجهر الضوئي والمجهر الإلكتروني الماسح. وكشفت الدراسة أنه بعد فحص سبعة عشر من طيور أبو قردان وجد أن تسعة فقط منهم (53%) مصابون بهذا الطفيلي. وتم تجميع إناث الطفيلي من تحت الطبقة المبطنة للجزء الغدي من معدة الطائر. ويتميز شكل أجسام إناث الطفيلي بأنها ملتوية في حلزونة ضيقة طولية تشكل لفتين أو ثلاث لفات للجسم. أما الذكور فتوجد متحركة في التجويف، وأحياناً على بطانة جدار المعدة. وأجسام الذكور لها شكل اسطواني خيطي، ولكل ذكر شوكتان غير متساويتان في الطول وغير متشابهتان في الشكل. ولهذه الديدان كبسولة فمية كيتينية سميكة الجدران، شكلها يشبه القارورة في الإناث، وأنبوية الشكل في الذكور. ويقع الفرج في الثلث الخلفي من جسم الأنثى. بالنسبة للبشرة فهي مخططة عرضياً بشكل حاد وبطول الجسم وبدون أجنحة أو أشواك. وتظهر كل شفة كاذبة بحافتين، الحافة الخارجية متموجة ولها ثلاثة زوائد منتفخة. ويحتوى الجزء المنخفض الذي يقع بين حافتي كل شفة كاذبة على ثلاث زوائد على شكل زهرة مموجة. وتظهر في الجزء الأمامي من الجسم تخطيطات البشرة بشكل مجعد ولديها العديد من الزوائد المثلثة الشكل المدببة. وتظهر أيضاً حليلة واحدة متموجة في شكلها، وزوائد عديدة تشبه الأزرار، وتركيب ببيضاوي الشكل مخروطي مخطط يبرز في وسطه تركيب يشبه القضيب الملتوي. أما قرب منتصف جسم الإناث فتظهر التخطيطات المستعرضة العميقة ذات الحواف المتموجة.