

## Ultrastructural and genetic characterization of the two *Ascaridia galli* and *A. columbae* from birds in Taif, Saudi Arabia

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**Abstract:** The topographic ultrastructure and genetic characterization of the two ascaridid nematode worms *Ascaridia galli* and *A. columbae* respectively collected from the domestic fowl *Gallus gallusdomesticus* and the wild dove *Columba liviapalastinae* from Taif, Saudi Arabia are described. Comparison of the two nematodes, either on ultrastructural or Genetic characteristics has revealed the possibility of differentiating the two nematodes. Scanning electron microscopy of adult *A. columbae* showed the presence of two wide cephalic alae, three globular lips the inner surface of each is covered with two triangular teeth (spone-like), while in *A. galli* the cephalic alae are absent, three lips are covered with an outer shrunken cuticular surface and their inner surfaces are covered with a thick and continuous cuticular plate or tooth. In addition to the cuticular ventral surface of the male tail of *A. galli* is covered with small cuticular knobs or vesicles while that of *A. clumbae* is covered with faint transverse striations without any vesicles. RAPD-PCR analysis has revealed the presence of differences between the two nematodes including a high polymorphism with the percentage (83%). A total of 50 and 42 different specific markers were detected for *A. columbae* and *A. galli* respectively. The markers obtained in the present study might be used for identifying, tracking and the lineage the two nematode worms. So, these markers might have potential applications of a successful control ascaridid nematode worms in poultry industry.

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### 1. Introduction:

Nematodes of the genus *Ascaridia* (Dujardin, 1845) infect many species of birds. These nematode species may cause serious and frequently fatal diseases in farm and exotic birds kept in captivity, including parrots (Kajerova *et al.*, 2004). It is well known that members of the genus *Ascaridia* have monoxenous (direct) life cycles, develop within the gastrointestinal tract of the definitive host, produce eggs that embryonate outside the host and have prominent pre - anal sucker encompassed by a cuticularized ring (Anderson, 2000). The genus *Ascaridia* includes several species most of which are considered synonymous Cram (1927) and Bhalerao (1935) and Kajerova *et al.* (2004). *Ascaridia galli* (Schrank, 1788) Freeborn, 1923 was synonymised with *A. lineata* Schneider, 1866; *A. perspicillus* Rudolphi, 1803 (Lalchandama, 2010). *Ascaridia galli* and *A. columbae* are common nematode parasites reported to infect domesticated as well as wild birds (Soulsby, 1982). *A. galli* is the most common nematode in all types of production systems and has a worldwide distribution. (Permin *et al.*, 1997; Ashenafi & Eshetu, 2004; Martin-Pacho *et al.*, 2005; Rabbi *et al.*, 2006; Abdelqader *et al.*, 2008). Schwartz (1925) attributed the confusion in identifying ascaridid nematodes firstly to the fact that

they are morphologically closely related and secondly because the tendency of most workers who are not systematists is to make host determination of parasites. Mutafova T (1976) studied the karyotype of both *Ascaridia galli* and *A. dissimilis* and found that both have similar number of chromosome  $2n=10$  in female worm and  $2n=9$  for males. Scanning electron microscopy and Molecular approaches are the most effective and accurate means for the detection of many organisms and for screening of genetic variation among populations (Wongsawad and Wongsawad, 2010). Molecular techniques based on genomics are very useful for epidemiological and diagnostic tools as well as for research on genetic variation of parasitic organisms (Mas-Coma *et al.*, 2005; Meshgi *et al.*, 2008; Tawfeek *et al.*, 2009). DNA Polymorphism assay based on random amplified Polymorphic DNA polymerase chain reaction (RAPD-PCR) has been proved useful for analyzing the inter- and intra-specific genetic variations and phylogenetic relationships (Gasser, 2005; Mas-Coma *et al.*, 2005; Nuchprayoon *et al.*, 2007; Rokni *et al.*, 2010; Awad *et al.*, 2010). The technique is very rapid, simple and generates reproducible fingerprints of the PCR products. In addition, it neither depends on previous knowledge or availability of the target DNA sequences nor requires

DNA hybridization (Mohammedzadeh *et al.*, 2007; Nuchprayoon *et al.*, 2007; Sripalwit *et al.*, 2007).

The aim of the present work is to define a highly reliable ultrastructural and genetic characterization of *Ascaridia galli* and *A. columbae* species to assess worm differentiation via molecular and morphometric characters using RAPD-PCR and scanning electronic microscopy (SEM).

## 2. Materials and methods

### 2.1- Samples collection

A total of 225 birds including 29 domestic chicken (*Gallus gallusdomesticus*) and 196 wild doves (*Columba liviapalaestinae*) were respectively collected from market and from wells at Taif city and its surroundings. Birds were brought to the lab dissected and examined for helminth parasites. The collected nematodes were identified as *Ascaridia galli* and *Ascaridia columbae*. Worms were fixed in 10% formalin solution or 3% glutaraldehyde in phosphate buffer PH 7.2 then CO<sub>2</sub> critical point dried and gold coated and examined in JEOL 6390 SEM at Taif university.

### 2.2- Genomic DNA extraction

Genomic DNA was extracted from two isolates using Wizard<sup>®</sup> SV Genomic DNA Purification System (Promega Madison, WI, USA) following the manufacturer's instructions.

### 2.3- RAPD PCR

PCR reactions were carried out with ten arbitrary 10-mer RAPD primers (Operon Tech., Inc.). PCR reactions were conducted using 2x superhot PCR Master Mix (Bioron; Germany) with 10 Pmol of each 10 different primers. The codes and sequences of these primers are listed in Table 1. The 25 µl reaction mixture was (10 Pmol. of each primer, 30-50 ng of DNA template and 12.5 µl of 2x superhot PCR Master Mix). The PCR protocol was initial denaturation, 94°C for 2.5 min and 35 cycles of

subsequent denaturation, 94°C for 45 s; annealing temperature, 36°C for 30 s; extension temperature, 72°C for 2 min and final extension, 72°C for 10 min. PCR products were electrophoresed on 10 x 14 cm 1.5% agarose gel electrophoresis with DNA ladder standard 100 bp (Jena Bioscience, Germany) for 30 min using Tris-borate- EDTA Buffer and visualized by ultraviolet Transilluminator after staining with 0.5 µg/ml ethidium bromide.

### 2.4- Data analysis

All gels were visualized and documented using a GeneSnap 4.00-Gene Genius Bio Imaging System (Syngene; Frederick, Maryland, USA). Each RAPD-PCR amplified DNA fragment was assumed to represent a single locus. The digital image files were analyzed using Gene Tools software from Syngene. The densitometric scanning of each based on its three characteristic dimensions was carried out. Each band was recognized by its length, width and intensity. Accordingly, the relative amount of each band was measured and scored.

## 3- Results

### 3.1- SEM Morphological analysis

Scanning electron microscopy of adult *A. columbae* showed the presence of two wide cephalic alae extending on both lateral sides of the body (Figs.1&2), three globular lips the, inner surface of each, is covered with two triangular teeth (spone-like) (Fig.3), while in *A. galli* the three lips are covered with an outer shrunken cuticular surface and their inner surfaces are covered with a thick and continuous cuticular plate or tooth (Fig.4). In addition to the cuticular surface of the male tail of *A. galli* is covered with small cuticular knobs or vesicles (Figs 5 & 6) while that of *A. clumbae* is covered with faint transverse striations without any cuticular vesicles (Figs. 7 -10).



Fig.1. *A. columbae* scanning electron micrograph of anterior extremity of the worm showing two lateral cephalic alae (wings).

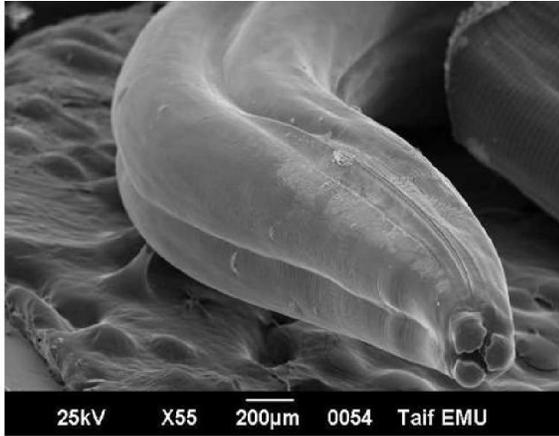


Fig.2. *A. columbae* anterior extremity of the worm showing three large trilobed lips and two lateral cephalic wings.

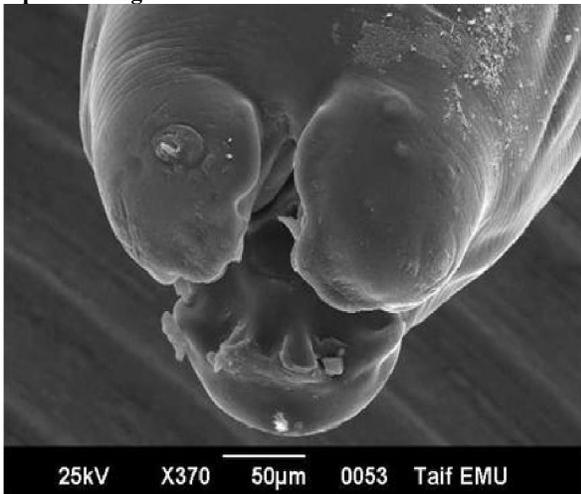


Fig.3. *A. columbae* mouth opening surrounded with three globular lips, notice the presence of two triangular (spoon like) teeth on the inner surface of each lip.

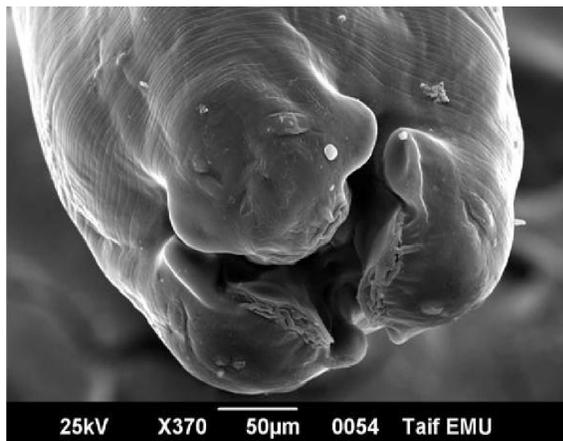


Fig.4. *A. galli* mouth opening surrounded with three large trilobed lips, the inner surface of each is covered with a smooth cuticular plate and the outer surface is covered with a corrugated cuticular band.

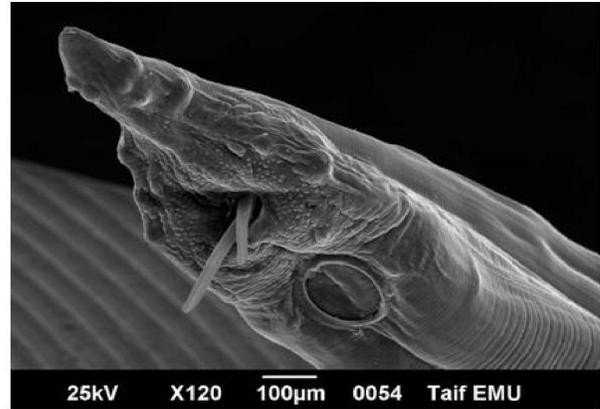


Fig. 5 *A. galli* male posterior e extremity showing the presence of cuticular vesicles in the ventral surface , posterior to the preloacal sucker, and cloacal papillae.

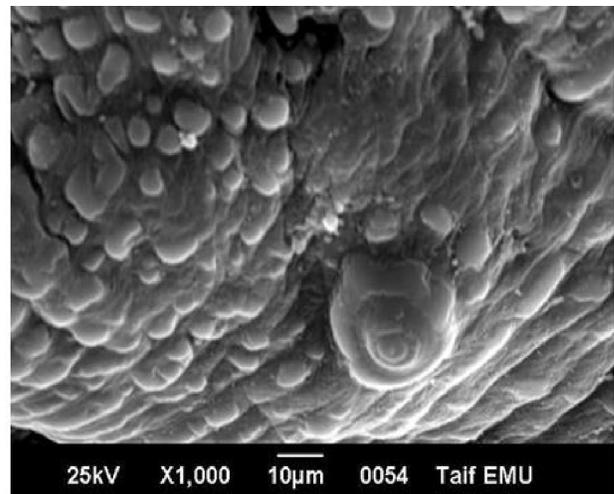


Fig.6. *A. galli* enlarged of figure 5 showing the cuticular vesicles and one genital papilla.

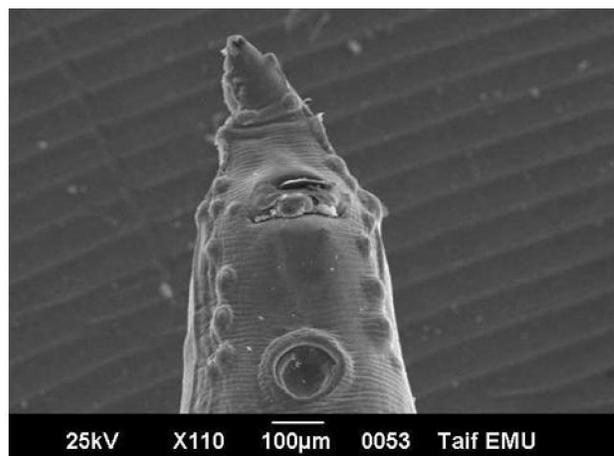


Fig. 7. *A. columbea* male posterior extremity showing absence of cuticular vesicles and presence of cuticular transverse striations .

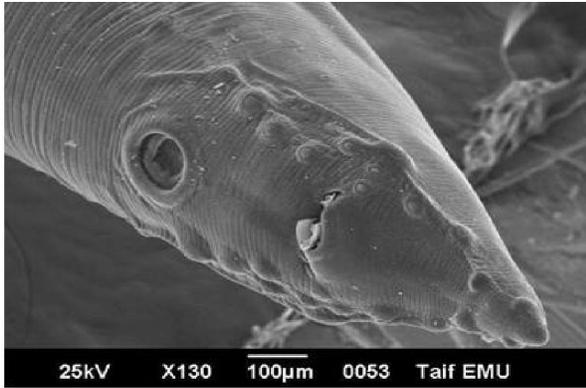


Fig. 8 *A. columbae* enlarged of figure 7 showing male cloacal papillae.

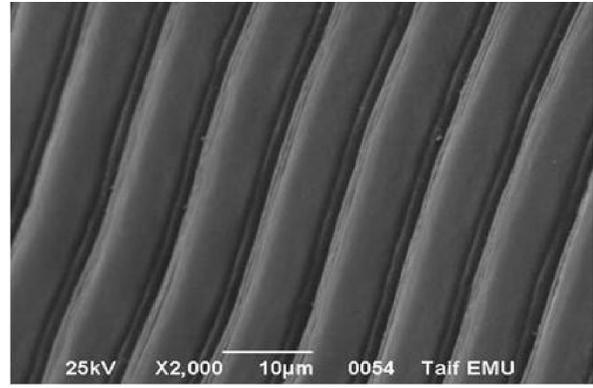


Fig.9. *A. columbae* SE micrograph of body surface ornamented with wide cuticular transverse striations.

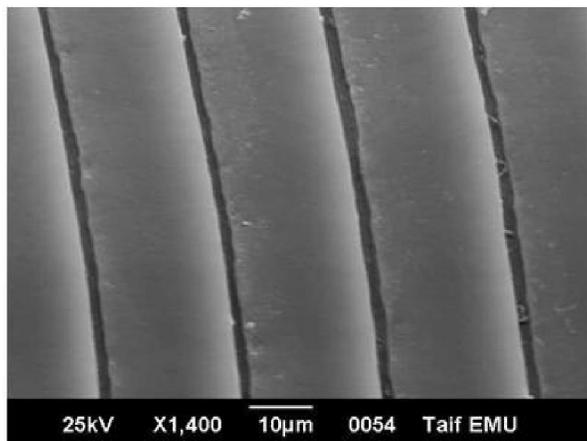


Fig.10. *A. galli* SE micrograph of body surface ornamented with wider cuticular transverse striations.

**3.2- RAPD-PCR analysis**

The two studied isolates were subjected to RAPD-PCR using ten primers (Figs. 11, 12 and 13). RAPD-PCR data were analyzed. Genetic profiles of the two studied isolates were characterized. Out of the 10

primers used, 8 (80%) successfully produced amplified DNA with consistently reproducible banding pattern(Figs. 11, 12 and 13). A distinct polymorphism was clearly obvious between to studied isolates in the used 8 primers

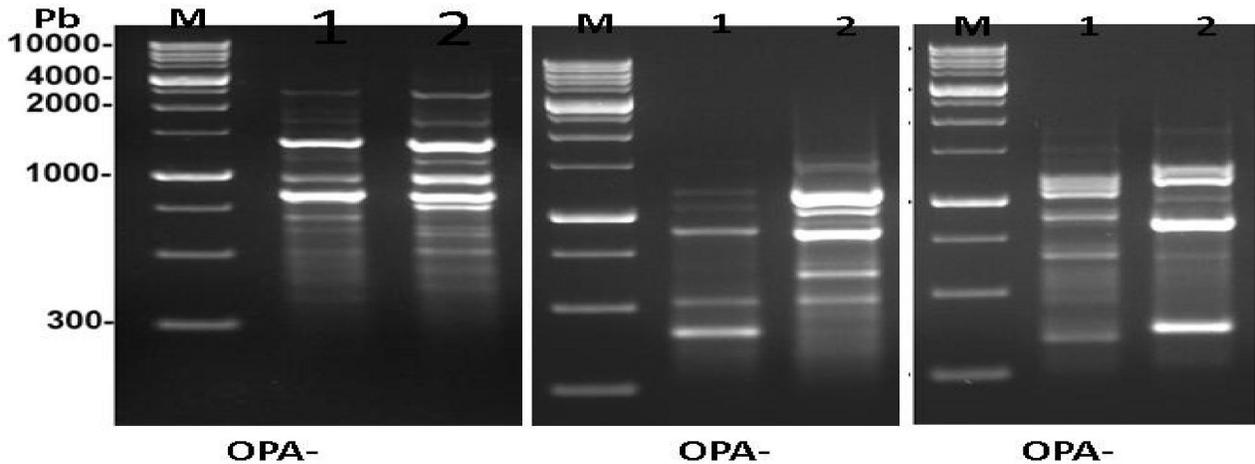


Fig. 11. : Electrophoretic banding pattern of RAPD-PCR products revealed from (1) *A. columbae* and (2) *A. galli* with OPA-02, OPA-03 and OPA-04

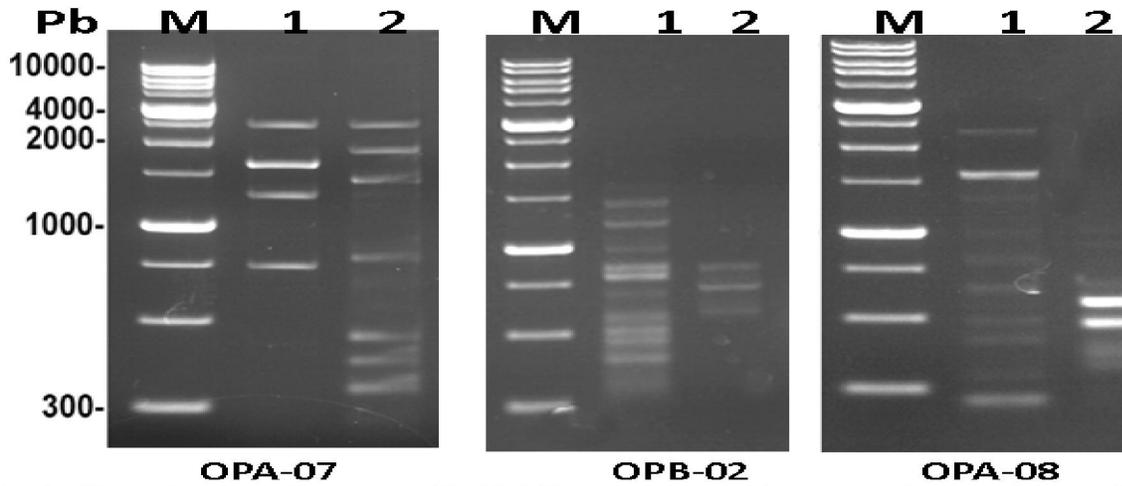


Fig. 12. : Electrophoretic banding pattern of RAPD-PCR products revealed from (1) *A. columbae* and (2) *A. galli* with OPA-07, OPB-02 and OPA-08

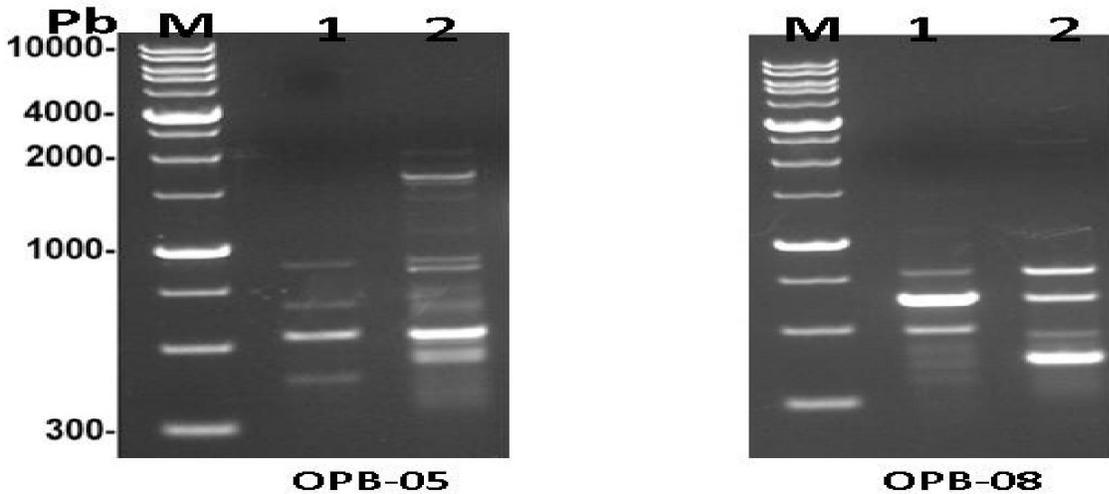


Fig. 13. : Electrophoretic banding pattern of RAPD-PCR products revealed from (1) *A. columbae* and (2) *A. galli* with OPB-05 and OPB-08

Table 1 shows the list of primers used, their nucleotide sequences and the total number of bands for each isolate produced by eight primers. The used primers have amplified 31 bands with 111 total number of bands, out of which 92 bands were polymorphic bands with heterogeneity percentage 83 % and 19 bands were monomorphic bands with homogeneity percentage (17%). The lowest polymorphism 50% was recorded with OPB-08 primer, on the contrary the highest one was 94.7 with OPA-02. The obtained banding patterns showed a distinct variation between the two examined isolates. Variations in the size and number of amplified fragments from each primer were detected. The size

of amplified fragments ranged from approximately 280 bp in primer OPA-08 to approximately 2500 bp in primer OPA-07. The maximum number (19 fragments) was amplified with primer OPA-02 and the minimum number (9 fragments) was amplified with primer OPA-07.

Table 1 shows the specific markers obtained across RAPD-PCR analysis. 50 RAPD markers were obtained specific for *A. columbae* and 42 for *A. galli*. Primer OPA-02, generated the highest number of specific markers. Twelve markers were generated from this primer. In contrast, OPB-02 and OPB-08 primers produced the lowest number of markers (two per primer).

**Table 1. Names and sequences of the used primers, amplified fragment range, number of total, polymorphic and monomorphic bands.**

Primer Code	Primer sequence	Amplified Fragment (bp)	Total bands	Polymorphic bands	Specific markers A. columbae	Specific markers A.galli	Monomorphic Bands (MW)	Polymorphis m%
OPA-02	TGCCGAGCT	350-2500	19	18	12	6	1250	94.7
OPA-03	AGTCAGCCAC	400-1500	14	12	4	8	550-1500	85.7
OPA-04	AATCGGGCTG	350-1800	15	10	7	3	450-600-1100-1250-1800	66.7
OPA-07	GAAACGGGTG	350-2500	9	8	3	5	2500	88.9
OPA-08	GTGACGTAGG	280-2300	15	12	7	5	500-600-1000	80
OPB-02	TGATCCCTGG	350-1600	14	13	11	2	550	92.9
OPB-05	TGCGCCCTTC	350-2100	15	14	3	11	850	93.3
OPB-08	GTCCACACGG	350-1400	10	5	3	2	400-450-600-750-1100	50
<b>Total</b>		280-2500	111	92	50	42	19	83

#### 4-Discussion

In the present study the two nematodes were compared and characterized. Their specific characteristics were established, including lips, cephalic papillae, body cuticle, spicules and caudal papillae of the male. Their DNA fingerprinting were detected including monomorphic and polymorphic bands, specific markers, homogeneity and heterogeneity. The present description of *Ascaridia galli* from Saudi Arabia is similar to the description given by Ashour (1994) in Egypt. In Saudi Arabia Ramadan and Znada (1992) and Dehlawi (2007) reported the presence of *A. galli* and described the life cycle of *Ascaridia* recovered from domestic fowl from Jeddah.

Kajerova *et al.* (2004) made a review and a key for ascaridia from different hosts and differentiated *A. galli* and *A. columbae* on the bases of spicule length and female tail length. However the present work using the scanning electron microscope has documented new clear differences between the two nematodes including the shape of teeth being spoon-shaped in *A. columbae* and continuous cuticular plate in *A. galli*, the ventral surface of the male cloacal area, being covered with small cuticular vesicles in *A. galli*, while these vesicles are absent in *A. columbae*, the outer cuticular surface of the lips is wrinkled in *A. galli*, while it is not in *A. columbae*. In addition to the presence of cervical wings in *A. columbae* and its absence in *A. galli*.

Both *A. galli* and *A. columbae* nematodes have been reported from different hosts and different parts all over the world (Fedynich, 2008).

The present work has added new characteristics to be used in differentiation of the two nematode. This includes the cephalic alae which is

wide in *A. columbae* compared to the narrow one of *A. galli*, the cuticular vesicles covering the ventral surface of the male tail of *A. galli* and its absence in *A. columbae*, the structure of the lips which are robust and globular in *A. columbae* while in *A. galli* lips are covered with a rather shrunken cuticle, and the teeth are triangular or spoon-like cuticular thickening covering the inner surface of each lip, while in *A. galli* teeth form a complete cuticular cover on the inner surface of each lip. The obtained polymorphic banding patterns from all studied RAPD-PCR primers confirmed that, the RAPD-PCR technique based on DNA from the two ascaridid nematodes *Ascaridia galli* and *A. columbae*, has demonstrated genetic variability amongst these Saudi isolates. The majority of random primers used gave distinctly reproducible patterns in the entire isolates studied. However, primers varied in the extent of information. These results demonstrate the random pattern of amplification, the heterogeneity and polymorphism of the *Ascaridiagalli* and *A. columbae* studied isolates. The obtained specific markers proved to be quite powerful in characterization of each isolate as well as distinguishing these different isolates. Further detailed investigations at DNA sequence level are needed to obtain complete and accurate identification of ascaridid nematode worms in KSA.

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