Parasitology International 71 (2019) 59–72

Contents lists available at ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

Morphological and molecular appraisal of cyclophyllidean cestoda parasite *Raillietina saudiae* sp. nov. infecting the domestic pigeon *Columba livia domestica* and its role as a bio-indicator for environmental quality

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ARTICLE INFO

Keywords:
Columba livia
Raillietina spp.
Morphological description
Molecular phylogenetic analyses
Biological indicators

ABSTRACT

Pigeons members of the order Columbiformes are a cosmopolitan group of birds with abundant and large populations associated with human activities. Therefore, the current study was conducted to determine the parasitic infections within the domestic pigeon *Columba livia domestica*. Eighteen pigeons were examined for the presence of gastrointestinal helminths, of which 77.78% were infected with a cyclophyllidean cestoda parasites. The morphology of this parasite based on light and scanning electron microscopic studies, revealed the presence of gravid worms, 2.00–4.52 cm long and 0.23–0.59 mm wide; a scolex had four suckers equipped by 5–6 rows of minute hooks and retractable rostellum with 230–250 hooks; genital pores unilateral; oval testes with 27–37 in number; bilobed ovary; post–ovarian vitelline gland; and 24–28 egg capsules present in uterus with 5–6 spherical eggs in each capsule. Molecular analysis based on sequences of ITS2 and ND1 gene regions was performed to confirm the taxonomy of this parasite based on its morphology. This revealed close identity of up to 92.0% and 72.0% for ITS2 and ND1 gene regions, respectively, with other cestoda species obtained from GenBank. Phylogenetic analysis supported the placement of this cyclophyllid species within Davaineidae with close relationships to the previously described species of *R. chiltoni*, *R. dromaius*, and *R. beveridgei* based on the ITS2 gene region and *R. coreensis* and *R. sonini* based on the ND1 gene regions. Heavy metals accumulation in the recovered parasite and its host showed significantly higher concentrations in the parasite compared to its host tissues. Generally, concentrations of metals exceeded the permissible limits recommended by the US Environmental Protection Agency. Therefore, pigeon cestodes can be regarded as useful bio-indicators when evaluating the environmental pollution of terrestrial ecosystems by heavy metals.

1. Introduction

Pigeons *Columba livia* Gmelin [1] are members of the order Columbiformes and represent cosmopolitan birds adapted to adverse conditions in different parts of the world [2,3]. They are bred as a source of food, as cultural and religious symbols, and also used for different experimental purposes [4,5]. Because of their close interaction with humans and other domestic and wild birds, they serve as potential reservoir of zoonotic parasites [6,7]. Parasitism is gradually being accepted as one of the major selective forces affecting avian life histories [8].

The poultry industry has been confronted with various parasitic disease of economic significance [9]. Like other domestic poultry species, pigeons have shown high prevalence of protozoan and gastrointestinal helminth infections [10]. Endo–parasitism is one of the most important forms of disease occurrence of different cestoda species in poultry [11]. The most important cestoda parasites of poultry are *Raillietina*, *Hymenolepis*, *Choanotaenia* and *Davainea* species [12]. The *Raillietina* group is obligate, comprised a large assemblage of over 200 known species, widely dispersed cestoda parasites of avian and mammalian hosts and display a wide range of body forms, life histories and host associations [13]. Helminth infections affect pigeons causing high rates of morbidity and mortality among them [14]. Young birds or squabs are severely affected, with the infection causing problems of malnutrition and malnourishment and ultimately leading to growth

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https://doi.org/10.1016/j.parint.2019.03.002
Received 4 December 2018; Received in revised form 28 February 2019; Accepted 2 March 2019
Available online 04 March 2019

retardation and high susceptibility to secondary infections [15]. The morphological criteria used to distinguish *Raillietina* spp. includes size and shape of the scolex, morphology of the rostellum (armed with either a single or double rows of hooks) and suckers (armed or unarmed), the position (unilateral or irregularly alternating) and number of genital pores per segment, and number of eggs within each egg capsule in gravid proglottids [16,17]. However, the morphological characters of *Raillietina* spp. show marked variations within and between species. Therefore, it is difficult to identify them based only on the morphology. Instead, molecular approaches in combination with morphological analyses, have become the most effective and accurate methods for the identification of cestoda *parasites*, studying the interrelationships between different genera and construction of phylogenetic trees [18–20]. There are many DNA regions such as internal transcribed spacer 2 (ITS2) in nuclear gene and nicotinamide adenine dinucleotide dehydrogenase subunit 1 (ND1) in mitochondrial gene that are useful for identification of helminths and discrimination between species with similar morphologies [21,22].

The effects of pollution on parasitism are variable, i.e. pollution may increase parasitism if the pollutant mainly affects the host rather than the parasite (immune–toxic chemicals), or a negative effects in certain parasites that are more susceptible to the particular pollutant than their hosts [23,24]. Interaction between parasites and pollution can be even more complex. In fact, some parasites may even have a "positive" influence on their hosts when exposed to the environmental pollution or, on the contrary, they may have synergistic effects becoming more harmful to the host or influencing gonad development of the host [25]. It has been postulated that parasitism may be advantageous where hosts are exposed to a polluted environment and parasites might be outweighed by the positive effect of reduced pollutant levels from its host [26–28].

However, there is lack of knowledge on the helminths that are affecting poultry in the fast-growing poultry industry in Saudi Arabia. This study designed to provide more information on the identification of *Raillietina* parasites of the domestic pigeon *C. livia domestica*, based on morphology and molecular analyses. In addition, the bioaccumulation effects of this parasite for heavy metals were studied to determine the use of *Raillietina* as bio-indicator.

2. Materials and methods

2.1. Collection of pigeons

A total of 18 specimens of the domestic pigeon *C. livia domestica* (Columbidae) (male and female with same age and body weight, $n = 9$ for each sex) were collected during the period of the current investigation from commercial poultry farms in Shaqraa Province, Riyadh, Saudi Arabia. The collected pigeons were transported immediately to the Laboratory of Parasitology Research at Zoology Department, College of Science, King Saud University, Riyadh, Saudi Arabia; dissected and necropsied, and each gastrointestinal tract was isolated. Worms were recovered from the small intestine and washed with normal saline.

2.2. Light microscopic examination

The recovered worms were preserved in hot alcohol–formalin–acetic acid (AFA) fixative, dehydrated in ascending grades of ethyl alcohol, then stained with acetic acid alum carmine, cleared in xylene and finally mounted in Canada balsam. Worms were identified according to the identification key of Yamagutii [29], and Soulsby [30]. Parasite prevalence of *C. livia* was calculated according to Bush et al. [31]. Mean intensity was calculated as number of parasites per infected pigeon. Photomicrographs of the adult worms were captured with the aid of microscope Leica DM 2500 (NIS ELEMENTS software, ver. 3.8).

2.3. Scanning electron microscopic examination

Cestoda parasites were fixed at 3% buffered glutaraldehyde [pH 7.2], washed for 4 h at 4 °C in buffered sodium cacodylate [pH 7.4], dehydrated in graded series of ethanol, infiltrated with amyl acetate, processed in a critical point dryer "LEICA, EM CPD300" with liquefied CO_2, and then sputter-coated with gold–palladium in Auto fine coater (JEOL, JEC–3000FC). Examination and photography of the prepared specimens were performed by using an Etec Autoscan at 10–kV JEOL scanning electron microscope (JSM–6060LV) in the Central Laboratory at King Saud University, Riyadh, Saudi Arabia. Measurements include the range followed in parentheses by the mean ± standard deviation. All measurements are in millimeters unless otherwise stated.

2.4. Molecular analysis

Genomic DNA was extracted from ethanol–preserved samples using a DNeasy tissue kit© (Qiagen, Hilden, Germany) following the manufacturer's information. The concentration and purification of genomic DNA was quantified with a NanoDrop ND–1000 spectophotometer (Thermo Fischer Scientific, Inc., Wilmington, DE, USA) and 20 ng genomic DNA was used for the polymerase chain reaction (PCR). Both ITS2 and ND1 gene regions were amplified using GeneJET™ PCR Purification Kit [Thermo (Fermentas)] following the manufacturer's protocol in a total volume of 50 µl, including 5 µl 10 × buffer, 5 µl of each dNTP (10 mM), 10 µl of each primer (1 pmol/ µl), 0.3 µl of Taq polymerase (5 U/ml), 2.5 µl $MgCl_2$ (50 mM), and 2 µl of total genomic DNA. For PCR amplification, primers for the ITS2 gene region were Forward 3S (5′–GGT ACC GGT GGA TCA CTC GGC TCG TG–3′) and Reverse BD2 (5′–TAT GCT TAA ATT CAG CGG GT–3′) designed by Morgan and Blair [32]. In addition, primers used for the ND1 gene region were Forward JB11 (5′–AGA TTC GTA AGG GGC CTA ATA–3′) and Reverse JB12 (5´–ACC ACT AAC TAA TTC ACT TTC–3′) designed by Barazesh et al. [33]. Cycling conditions were: 5 min initial denaturation at 94 °C, followed by 35 cycles of 30 s DNA denaturation at 94 °C, 30 s primer annealing at 55 °C, 2 min extension at 72 °C, and a final extension at 72 °C for 7 min. PCR products were verified on 1% agarose gel in 1 × Tris–acetate–EDTA stained with ethidium bromide and visualized with UV trans–illuminator. The obtained bands of the expected size were gel–excised, purified and cloned using a PureLink™ Quick Gel Extraction Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Nucleotide sequencing was performed on a 310 Automated DNA Sequencer (Applied Biosystems, Foster City, CA) using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). A BLAST search was carried out to identify related sequences in the NCBI database (www.ncbi.nih.gov/BLAST/). Sequences were aligned directly using CLUSTAL–X multiple sequence alignment with cestoda parasite sequences obtained from GenBank. The alignment was corrected manually using the alignment editor BIOEDIT 4.8.9 software [34]. Phylogenetic and evolutionary analyses were conducted for each gene using MEGA version 6.0. The data were analysed using neighbour–joining (NJ) and maximum–likelihood (ML) methods. The statistics supported for branches were tested using 1000 bootstrap replicates. Each tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Evolutionary distances were computed using the p–distance method.

2.5. Chemical analysis

Pigeon and parasite tissues were analysed to detect heavy metals, according to the procedure described by UNEP/FAO/IOC/IAEA [35]. Samples were digested with concentrated nitric acid and perchloric acid (2:1 *v*/v) at 60 °C for 3 days. After digestion, samples were diluted with deionized water and then analysed for trace elements in inductively coupled plasma–optical emission spectroscopy (ICP–OES). Values of all monitored heavy metals were presented in mg/g wet–weight, comparing with values detected by FAO/WHO [36]. The absorption wavelengths and detection limits for the measurable heavy metals were 188.980 nm and 0.16 ppm for Cd, 267.716 nm and 0.04 ppm for Cr, 327.395 nm and 0.05 ppm for Cu, 257.610 nm and 0.05 ppm for Mn, 220.353 nm and 0.13 ppm for Pb, 213.857 nm and 0.04 ppm for Zn, 231.604 nm and 0.001 ppm for Ni, 238.204 nm and 0.01 ppm for Fe, 279.553 nm and 0.01 ppm for Mg, 396.152 nm and 0.12 ppm for Al, 238.892 nm and 0.02 ppm for Co, and 202.032 nm and 0.05 ppm for Mo, respectively. All values from chemical analyses were presented as mean ± SE. Data obtained from the experiment were subjected to one-way analysis of variance (ANOVA) test followed by Duncan's multiple range test. Statistical analysis was performed using SPSS v.18 software package (SPSS, Chicago, Illinois, USA). *P* values ≤.05 were considered statistically significant.

3. Results

Fourteen out of 18 specimens of both sexes of the domestic pigeon's *C. livia* domestica were found to be naturally infected with cestoda parasites in the posterior parts of the small intestines, yielding a prevalence of 77.78% with intensity of 10–14 (12) cestoda/infected pigeon. The infection rate was quite similar in both female and male pigeons.

Description (Figs. 1A-K, 2A-F, 3A-G); for principal measurements, see Table 1).

Strobila measured 2.00–4.52 (3.54 ± 0.01) cm long, with maximum width of 0.23–0.59 (0.47 ± 0.01). Scolex containing four suckers and rostellum (Figs. 1A-C, 2A, 3A). Scolex 0.189–0.325 (0.266 ± 0.01) long, 0.165–0.183 (0.176 ± 0.01) wide. Suckers are rounded, 0.069–0.088 (0.073 ± 0.001) long and 0.031–0.044 (0.039 ± 0.001) wide, and armed marginally with 5–6 rows of minute hooks 0.005–0.008 (0.007 ± 0.001) long (Figs. 1A,B,E, 2A,E, 3A,C). Rostellum measured 0.051–0.076 (0.066 ± 0.001) long and 0.07–0.094 (0.084 ± 0.01) wide, with double crown of alternating hammer–shaped hooks (Figs. 1D, 2B,C, 3A,B). Rostellar hooks 230–250 (240) in number, with length of 0.0136–0.0153 (0.0149 ± 0.001) long and width of 0.0087–0.0098 (0.0092 ± 0.001). Each hook consisted of a blade, a guard and a handle (Figs. 1A-E, 2D, 3B,C). Neck was absent. The remaining part of strobili was a craspedote and consisted of 90–140 (120) proglottids: 25–43 (32) immature, 17–27 (22) mature, and up to 36 (51) gravid proglottids (Fig. 1A).

Mature proglottids measured 1.32–2.31 (1.91 ± 0.01) long by 0.09–1.42 (1.21 ± 0.01) wide. Cirrus sac pyriform 0.166–0.189 (0.173 ± 0.01) long, 0.094–0.120 (0.112 ± 0.01) wide, barely reaching to the excretory vessels, protrusible with duct capable of evagination and densely covered with minute spines (Figs. 1F,G, 3D,E). Genital pores unilateral and marginal, situated in the anterior half of the proglottid (Figs. 1H,I, 3D). The genital ducts passed between the excretory vessels (Fig. 1J). Vas deferens much coiled and run parallel to the anterior margin of the segment to the level of the ovary and surrounded with prostatic cells (Fig. 3D,E). Testes oval in shape, 27–37 in number, 0.046–0.071 (0.067 ± 0.001) long, 0.023–0.041 (0.037 ± 0.001) wide, and situated antero-dorsally on each side of the ovary; 17–21 in aporal side and 11–16 poral side; and extend laterally to the ventral excretory vessels (Figs. 1H, 3D). Bilobed ovary located in

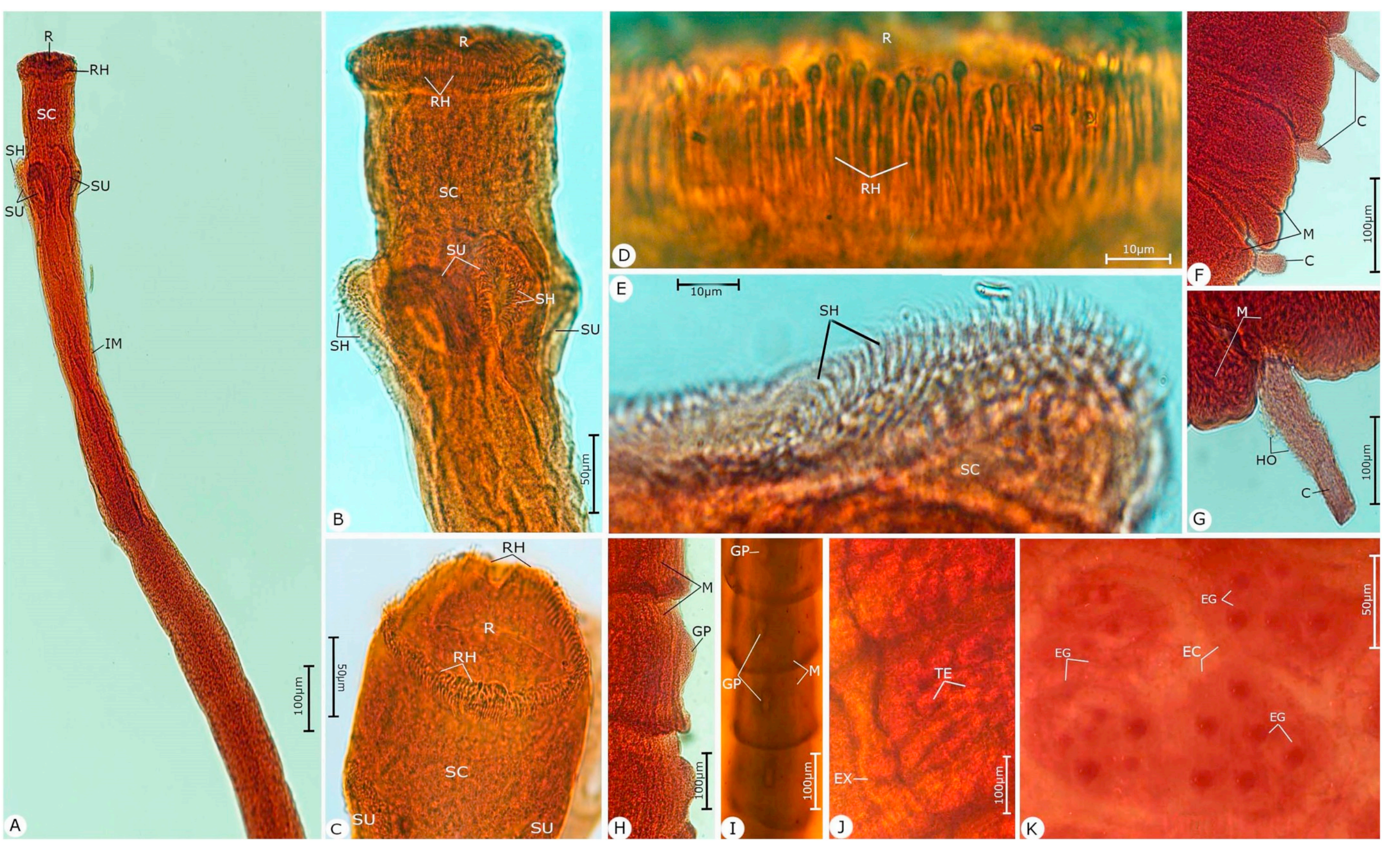

Fig. 1. (A–K) Photomicrographs of the adult cestoda parasite *R. saudiae* sp. nov. infecting *Columba livia domestica*. (A) Anterior extremity of the adult worm with scolex (SC) had rounded suckers (SU) armed with sucker hooks (SH) and well distinct rostellum (R) with two rows of rostellar hooks (RH), followed by immature proglottids (IM). (B—K) High magnifications for different body parts of: (B,C) Anterior extremity showing scolex (SC) bearing four suckers (SU) armed with sucker hooks (SH) and rostellum (R) armed with rostellar hooks (RH). (D) Rostellar hooks (RH) on rostellum (R). (E) Sucker (SU) armed with sucker hooks (SH). (F,G) Evaginated cirrus (C) from genital pores in mature proglottids (M) and densely covered with minute hooks (HO). (H,I) Unilateral genital pores (GP) on mature proglottids (M). (J) Mature (M) proglottids with testes (TE) and excretory canal (EX). (K) Egg capsules (EC) filled with eggs (EG).

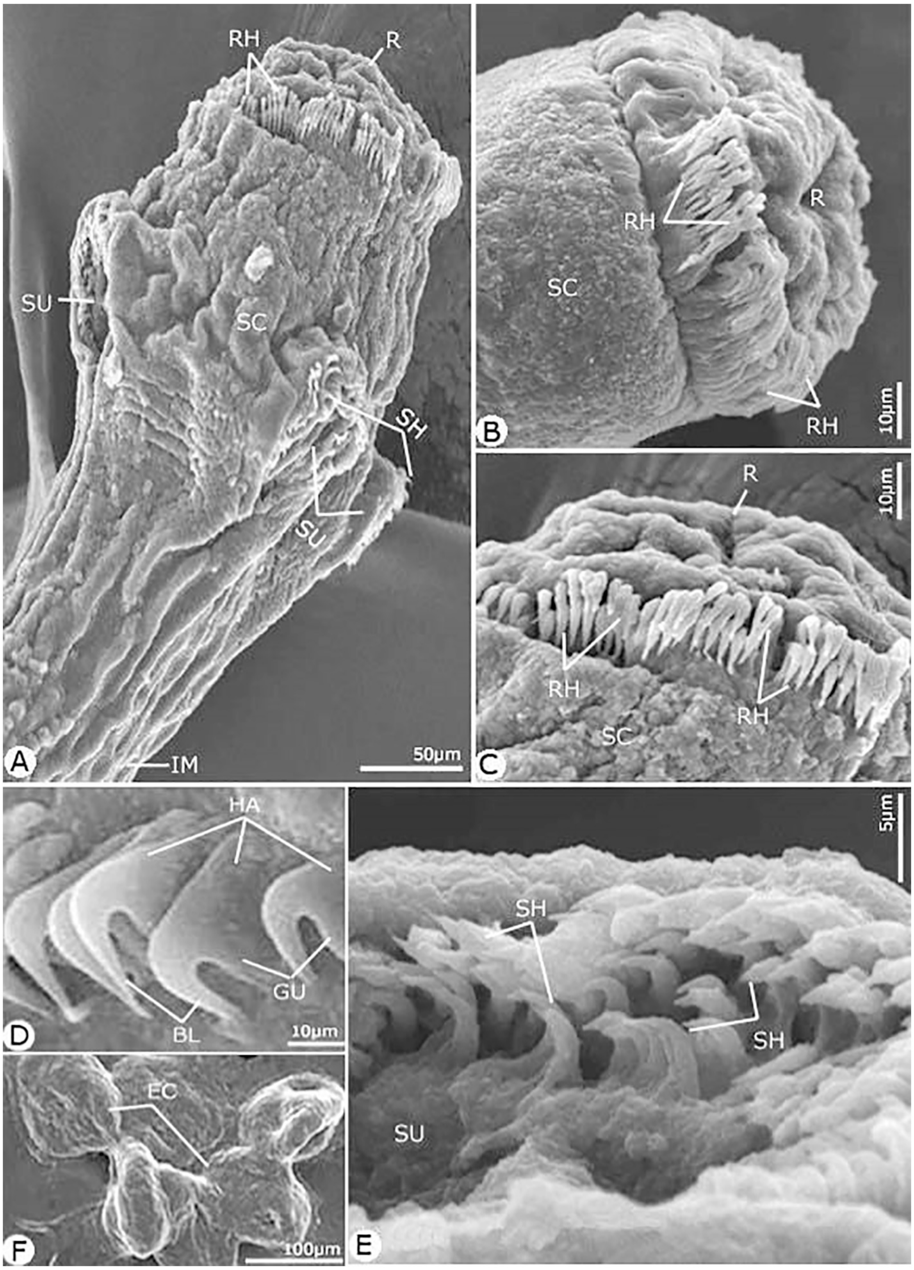

Fig. 2. (A–L) Scanning electron micrographs of *R. saudiae* sp. nov. infecting *Columba livia domestica*. (A) Anterior extremity of the adult worm with scolex (SC) had rounded suckers (SU) armed with sucker hooks (SH) and well distinct rostellum (R) with two rows of rostellar hooks (RH), followed by immature proglottids (IM). (B–E) High magnifications for different parts of the anterior extremity showing: (B,C) Scolex (SC) bearing rostellum (R) armed with rostellar hooks (RH). (D) Hammer–shaped rostellar hooks; each one consisted of handle (HA), guard (GU), and blade (BL). (E) Sucker (SU) on scolex (SC) armed with 5–6 rows of sucker hooks (SH). (F) Egg capsules (EC).

the middle of proglottid, 0.017–0.019 (0.018 ± 0.001) long, and 0.012–0.014 (0.013 ± 0.01) wide (Fig. 3D). Vitelline gland irregularly dentiform and situated behind the ovary (Fig. 3D). Gravid proglottids wider than longer, 0.14–2.53 (1.78 ± 0.01) long, 1.65–2.87 (2.32 ± 0.01) wide (Fig. 3F). Egg capsules subglobular or ovoid in shape filled the uterus and presented as 24–28 (26) sets in each proglottid (Figs. 1K, 2F, 3G). Each capsule contained 5–6 spherical eggs (Fig. 1O, 3G). Each egg 0.020–0.029 (0.024 ± 0.001) long by 0.017–0.023 (0.019 ± 0.001) wide and surrounded by a thin membrane (Fig. 3G).

3.1. Taxonomic summary

Parasite name:*Raillietina saudiae* sp. nov. (Davaineidae).

Type-host: Domestic pigeon *Columba livia domestica* (Columbidae).

Site of infection: Posterior part of the small intestine of infected pigeon.

Specimens studied: The description based on 15 specimens prepared as follows: whole mounts of 10 entire worms, 8 detached mature proglottids and 5 detached gravid proglottids, serial cross-sections of 2 detached mature proglottids, 5 worms and 3 detached mature proglottids examined with SEM.

Five complete specimen plus fragments of two additional mature specimens, five gravid specimens, and 10 specimens prepared for SEM.

Type-locality: Sharqaa Province, Riyadh, Saudi Arabia.

Prevalence: 77.78%.

Intensity of infection: 10–14 (12).

Specimens deposited: Holotype (ten slides, including hand–cut transverse sections, and fragments in ethanol). Paratype (ten slides and fragments in ethanol), from the same host individual as the holotype. All samples were deposited in the parasitological collection of Zoology Department, College of Science, King Saud University, Saudi Arabia (Deposition no. 437/203–514).

Morbidity and mortality: Infected domestic pigeons were generally symptomless externally.

Etymology: The name of the new species refers to the locality

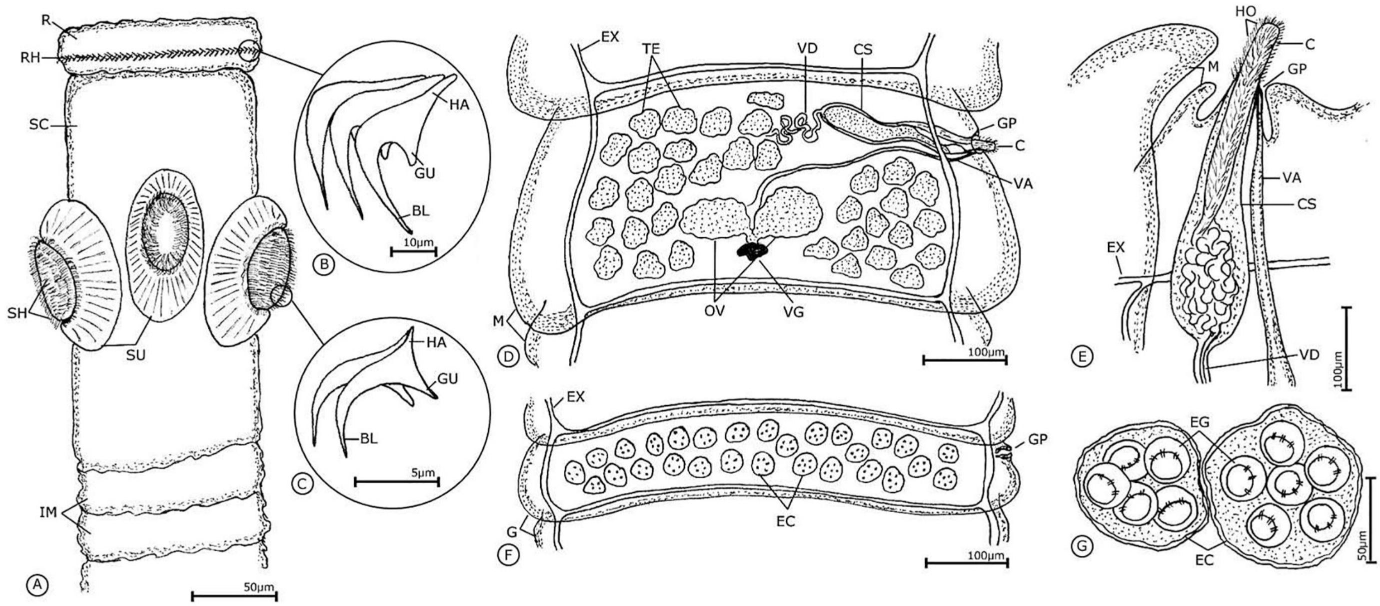

Fig. 3. (A-G) Line drawing for *R. saudiae* sp. nov. infecting *Columba livia domestica* showing different body parts. (A) Anterior extremity of the adult worm with scolex (SC) had rounded suckers (SU) armed with sucker hooks (SH) and well distinct rostellum (R) with two rows of rostellar hooks (RH), followed by immature proglottids (IM). (B) Rostellar hooks (RH). (C) Sucker hooks (SH). (D) Mature (M) proglottids with bilobed ovary (OV), testes (TE), vas deferens (VD), cirrus sac (CS), cirrus (C), vitelline gland (VG), excretory canal (EX), and genital pore (GP). (E) Protrusible cirrus (C) covered with minute hooks (HO) from cirrus sac (CS) within genital pore (GP) in mature proglottid (M), note the presence of vas deferens (VD) and excretory canal (EX). (F) Gravid proglottids (G) with egg capsules (EC) and observable genital pore (GP). (G) Egg capsules (EC) filled with eggs (EG).

where pigeons were recovered.

3.2. Remarks

Table 1 showed comparison between the current *Raillietina* species with those described previously of the domestic pigeons. The present *Raillietina* species was closely related to *R. Micracantha* Fuhrmann [40], *R. clerici* Fuhrmann [42], *R. tunetensis* Joyeux and Baer [46], *R. bungoensis* Sawada and Kugi [53], and *R. tetragona* Kassem [55] with a similar number of suckers hooks (5–6). The present species was similar to some comparable species, such as *R. crassula* Rudolphi [37], *R. torquata* Meggitt [43], *R. nripendra* Sharma [50], *R. tokyoensis* Sawada [51], *R. canabia* Magzoub, Kasim and Shawa [52], *R. zahratis* Magzoub, Kasim and Shawa [52], and *R. tetragona* Albaladejo, Acosta and Alonso [54], with regard to the unilateral position of the genital pore with anterior location to the middle portion of the segment. The other main differential feature is the shape and arrangement of rostellar hooks which appear in two rows with different number of hooks; 230–250 in the present species compared to 300 in *R. spiralis* Basczynska [41], 100 in *R. torquata*, 220 in *R. nagpurensis*, 120 in *R. horkei* Joyeux and Houdemer [45], 180 in *R. tunetensis*, 200 in *R. taiwanensis* Yamaguti [48], 10 in *R. kantipura* Sharma [50], and 220 in *R. tokyoensis*. In addition, the number of the testes per mature segment of *Raillietina* species was 27–37 in the present *Raillietina* species, 30–40 in *R. crassula*, 22–30 in *R. bonini*, 8–12 in *R. cryptacantha*, 19–30 in *R. Micracantha*, 24–28 in *R. clerici*, 20–30 in *R. torquata*, 19–22 in *R. nagpurensis*, 30 in *R. horkei*, 24–28 in *R. tunetensis*, 45–60 in *R. joyeuxi* Lôpez-Neyra [47], 14–17 in *R. taiwanensis*, 12 in *R. weissi*, 18–24 in *R. nripendra*, 45–50 in *R. kantipura*, 20–23 in *R. tokyoensis*, 28–34 in *R. bungoensis*, and 23–30 in *R. tetragona*. The present species was similar in the characteristic feature of bilobed ovary with *R. torquata* and *R. bungoensis*; while it differs from *R. nagpurensis*, *R. micracantha*, *R. spiralis*, and *R. tetragona* which have monolobulated ovary. In addition, it differs from *R. spiralis* and *R. echinobothrida* Al–Marsomy and Al–Hamadaani [56] in the position of the genital pore in the posterior location to the middle portion of each segment. Further, the number of eggs per egg capsule in the gravid proglottids of *Raillietina* species was 5–6 in the present *Raillietina* species vs. 1 in *R. crassula*, 4–6 in *R. spiralis*, 6–10 in *R. cylonica*, 6–12 in *R. torquata*, 3–8 in *R. nagpurensis*, 4–8 in *R. horkei*, 6–8 in *R. tunetensis*, 3–8 in *R. taiwanensis*, 6 in *R. weissi*, 4–9 in *R. nripendra*, 6–8 in *R. tokyoensis*, 3–9 in *R. zahratis*, 6–12 in *R. tetragona*, 4–12 in *R. tetragona*, and 8–12 in *R. echinobothrida*.

3.3. Molecular analysis

A total of 726 bp of the ITS2 gene region for *Raillietina saudiae* sp. nov. deposited in GenBank (gb| MK201802.1), with GC content of 44.2%. In the case of the ND1 gene region, sequence derived from the current *Raillietina* species was 508 bp with 32.5% GC content deposited in GenBank (gb| MK213271.1). Pairwise comparison of the isolated genomic sequences from the present *Raillietina* species with a variety of alternative class species and genotypes disclosed unique genetic sequences. Phylogenetic relationships were constructed using NJ and ML methods. Comparison of these novel genetic sequences with others retrieved from GenBank demonstrated a high degree of similarity, up to 92.0% and 72.0% for ITS2 and ND1 gene regions, respectively (Table 2).

For the ITS2 gene region, the present cestoda species yielded the highest BLAST scores with lowest divergence values for *R. chiltoni* (gb| AY382319.1), *R. dromaius* (gb| AY382320.1), *R. beveridgei* (gb| AY382318.1), *R. cesticillus* (gb| KP893422.1), *R. echinobothrida* (gb| MH122787.1, JN797628.1), and *R. tetragona* (gb| MH122788.1). A phylogenetic tree computed automatically by comparing the ITS2 query sequence of *R. saudiae* sp. nov. with those of other Cyclophyllidean cestodes available in GenBank showed that this order is represented by six families, which are Taeniidae, Mesocestoididae, Hymenolepididae, Davaineidae, Anoplocephalidae, and Dilepididae (Fig. 4). In this analysis, Davaineidae formed at sister group to Taeniidae and Hymenolepididae with strong nodal support. Also, the current analysis recovered a clade including Davaineidae with monophyly. Our phylogenetic analysis, incorporating new and existing data, investigated the placement of the examined davaineid species within Cyclophyllidea. It was

Table 1

Main morphological features and measurements of *Raillietina saudiae* sp. nov. compared with previous studies on *Columba* species on various geographical regions.

Comparable species (reference)	Host species (Locality)	Body size	Measurements for different body parts				
			Scolex	Sucker	Sucker hooks	Rostellum	Rostellar hooks
Raillietina crassula	*C. livia domestica* (Europe)	2.50–4.00 × 0.04	0.23	0.09	–	0.07–0.09	0.01–0.02
Raillietina bonini	*C. palumbus* (Europe)	0.98 × 0.077	0.165 × 0.189	0.105 × 0.055	–	0.01 × 0.05	–
Raillietina cryptacantha	*C. delegorguei sharpie* (Egypt)	1.20 × 1.5–0.02	–	0.035–0.04	0.006–0.007	0.04–0.059	0.0072–0.0009
Raillietina micracantha	*C. livia* (Tenerife)	1.0–1.5 × 0.02	0.200 ± 0.045 (0.158–0.243) × 0.247 ± 0.096 (0.192–0.391)	0.056 ± 0.0012 (0.043–0.071) × 0.039 ± 0.005 (0.033–0.048)	–	0.060 ± 0.001 (0.0058–0.0061) × 0.11-0 ± 0.001 (0.089–0.128)	0.0148 ± 0.001 (0.0132–0.0016) × 0.009-1 ± 0.0009 (0.0081–0.0102)
Raillietina spiralis	*Columba spp.* (India)	0.30–0.40 × 0.0128	0.224	0.052	–	0.15	0.0156
Raillietina ceylonica	*C. leuconata* (India)	0.30–0.40 × 0.0132	0.4	0.13	–	0.052	0.01
Raillietina clerici	*C. livia* (Urai)	2.12 × 0.15	0.2–0.3	0.075–0.08	0.008–0.009	0.05–0.06	0.008–0.01
Raillietina torquata	*C. livia* (India)	2.50–3.35 × 0.17–0.40	0.08–0.1	0.09–0.11	0.006–0.008	0.05–0.06	0.006–0.008
Raillietina nagpurensis	*C. livia domestica* (India)	2.50–2.74 × 0.19	0.339–0.382	0.142	–	0.216–0.241	0.017–0.019
Raillietina korkei	*C. livia* (India)	3.00 × 0.03	0.2	0.10	–	0.085 × 0.120–0.130	0.017–0.02
Raillietina korkei	*C. livia* (India)	1.64 × 0.02	0.2	0.06–0.07	0.01	0.13	0.018–0.020
Raillietina tunetensis	*C. livia domestica* (India)	0.30–2.10 × 0.015	0.2–0.3	0.075–0.08	0.008–0.009	0.05–0.06	0.008–0.009
Raillietina joyeuxi	*C. livia domestica* (Spain)	1.50–1.80 × 0.02	0.255–0.26	0.07 × 0.052	–	0.135	0.019–0.025
Raillietina taiwanensis	*C. livia domestica* (Formosa)	1.70 × 0.18	0.2–0.28	0.06–0.084	0.008–0.01	0.15–0.18	0.019
Raillietina weissi	*C. livia domestica* (France)	1.42 × 0.02	0.15–0.17	0.044–0.06	0.01	0.1	0.019
Raillietina nripendra	*C. livia* (Nepa)	2.00–2.50 × 0.125	–	0.062	–	0.11	0.012
Raillietina kantipura	*C. livia* (Nepal)	1.60–1.80 × 0.085	0.22	0.055	0.004	0.12	0.022
Raillietina tokyoensis	*C. livia domestica* (Japan)	1.00–1.30 × 0.025	0.09	0.04–0.048	–	0.074–0.089	0.008
Raillietina canabia	*C. livia* (Saudi Arabia)	1.30–2.00 × 0.046	0.28	0.06–0.07	–	0.13–0.15	0.014–0.017
Raillietina zahratis	*C. livia* (Saudi Arabia)	1.70–3.00 × 0.10–0.153	0.16–0.19	0.03–0.05	–	0.06	–
Raillietina bungoensis	*C. livia domestica* (Otia)	1.20–1.70 × 0.14–0.17	0.26 × 0.4	0.08–0.10	0.0075–0.010	0.12 × 0.27	0.028–0.030
Raillietina tetragona	*C. livia domestica* (Paloma)	1.50–4.15 × 0.14	0.165–0.280	0.082–0.114 × 0.052–0.080	0.006–0.008	0.058–0.090	0.0065–0.008
Raillietina tetragona	*C. livia domestica* (Libya)	3.00 × 0.1–0.15	–	–	–	–	–
Raillietina echinobothrida	*C. livia* (Iraq)	3.37	0.057 × 0.046	0.013	–	0.0025 × 0.027	–
Raillietina saudiae sp. nov.	*C. livia domestica* (Saudi Arabia)	3.54 ± 0.01 (2.00–4.52) × 0.47 ± 0.01 (0.23–0.59)	0.266 ± 0.01 (0.189–0.325) × 0.176 ± 0.01 (0.165–0.183)	0.073 ± 0.001 (0.069–0.088) × 0.039 ± 0.001 (0.031–0.044)	0.007 ± 0.001 (0.005–0.008)	0.066 ± 0.001 (0.051–0.076) × 0.084 ± 0.001 (0.073–0.094)	0.0149 ± 0.001 (0.0136–0.0153) × 0.009-2 ± 0.001 (0.0087–0.0098)

Comparable species (reference)	Measurements for different body parts				Distinctive features	References
	Testes	Cirrus pouch	Ovary	Egg size		
Raillietina crassula	–	0.1	–	–	2 rows of rostellar hooks with no. 60–70; testes no. 30–40	Rudolphi [37]
Raillietina bonini	0.04	0.205	0.23	–	Testes no. 22–30	Megnin [38]
Raillietina cryptacantha	–	0.09–0.165 × 0.058–0.08	–	0.02	6–8 rows of sucker hooks; number of rostellar hooks is 150–170; number of testes is 8–12;	Fuhrmann [39]
Raillietina micracantha	0.043 ± 0.006 (0.033–0.056) × 0.036 ± 0.007 (0.025–0.048)	0.077 ± 0.005 (0.066–0.084) × 0.030 ± 0.004 (0.028–0.040)	–	0.028 ± 0.003 (0.023–0.030) × 0.021 ± 0.004 (0.015–0.030)	5 rows of sucker hooks; rostellar hooks no. 150–200; testes no. 19–30	Fuhrmann [40]
Raillietina spiralis	0.039	0.101	0.352	0.104	7 rows of sucker hooks; rostellar hooks no. 300; 4–6 eggs in egg pouch	Basczynska [41]
Raillietina ceylonica	–	0.13 × 0.0312	–	0.028	Unilateral position of genital pore is posterior to middle portion of each segment margin; two rows of rostellar hooks with no. 120; few number of testes; 6–10 eggs in egg pouch	Basczynska [41]
Raillietina clerici	0.05	0.17 × 0.08	–	–	5–6 rows of sucker hooks; rostellar hooks no. 400; testes no. 24–28	Fuhrmann [42]
Raillietina torquata	–	–	0.057–0.063	0.017–0.02	8–11 rows of sucker hooks; 2 rows of rostellar hooks with no. 100; position of genital pore is anterior to middle portion of segment margin; testes no. 20–30; 6–12 eggs in egg pouch	Meggitt [43]
Raillietina nagpurensis	–	0.09–0.111 × 0.03–0.042	0.645	0.05–0.043	1 row of sucker hooks; rostellar hooks no. 220; 2 rows of rostellar hooks; testes no. 19–22; 3–8 eggs in egg pouch	Moghe [44]

Table 1 (*continued*)

Comparable species (reference)	Measurements for different body parts				Distinctive features	References
	Testes	Cirrus pouch	Ovary	Egg size		
Raillietina korkei	0.035	0.110	–	0.014 × 0.018	2 rows of rostellar hooks with no. 120; testes no. 30; 4–8 eggs in egg pouch	Joyeux and Houdemer [45]
Raillietina korkei	0.035	0.105–0.110 × 0.05	0.018 × 0.014	–	2 rows of rostellar hooks with no. 150–160; testes no. 24; 6–9 eggs in egg pouch	Joyeux and Houdermer [45]
Raillietina tunetensis	–	0.17 × 0.08	–	–	5–6 rows of sucker hooks; 2 rows of rostellar hooks with no. 180; testes no. 24–28; 6–8 eggs in egg pouch	Joyeux and Baer [46]
Raillietina joyeuxi	–	0.2–0.23 × 0.09–0.1	–	–	2 rows of rostellar hooks with no. 10; testes no. 45–60	Lôpez-Neyra [47]
Raillietina taiwanensis	–	0.1–0.02 × 0.028–0.042	0.036–0.042	–	Several rows of sucker hooks; rostellar hooks no. 200; 2 rows of rostellar hooks; testes no. 14–17; 3–8 eggs in egg pouch	Yamaguti [48]
Raillietina weissi	0.06–0.08	0.1–0.13 × 0.025–0.04	–	–	6–7 rows of sucker hooks; number of testes is 12; eggs in egg pouch are 6;	Joyeux and Baer [49]
Raillietina nripendra	–	0.12 × 0.055	–	–	1–3 rows of sucker hooks; rostellar hooks no. 150–180; position of genital pore is anterior to middle portion of segment margin; testes no. 18–24; 4–9 eggs in egg pouch	Sharma [50]
Raillietina kantipura	–	0.11 × 0.084	–	–	3 rows of sucker hooks; 2 rows of rostellar hooks with no. 10; testes no. 45–50	Sharma [50]
Raillietina tokyoensis	–	0.134–0.149	–	0.013	7–8 rows of sucker hooks; 2 rows of rostellar hooks with no. 220; position of genital pore is anterior to middle portion of segment margin; testes no. 20–23; 6–8 eggs in egg pouch	Sawada [51]
Raillietina canabia	–	0.10–0.13	–	0.04	Two rows of hammer-shaped rostellar hooks; unilateral genital pore opening	Magzoub, Kasim and Shawa [52]
Raillietina zahratis	–	0.19–0.21	–	0.065	Two rows of hammer-shaped rostellar hooks; unilateral genital pore opening; 3–9 eggs in egg pouch	Magzoub, Kasim and Shawa [52]
Raillietina bungoensis	0.055–0.065 × 0.04-5	–	0.255–0.270	0.03	5 rows of sucker hooks; testes no. 28–34	Sawada and Kugi [53]
Raillietina tetragona	–	0.134–0.140	0.065–0.110	–	Ovoid-shaped scolex and sucker; 10–11 rows of sucker hooks; unilateral genital pore opening with anterior location to middle portion of segment margin; one row of hammer-shaped rostellar hooks; with no. 105–115; testes no. 23–30; 6–12 eggs in egg pouch	Albaladejo, Acosta and Alonso [54]
Raillietina tetragona	–	–	–	–	Ovoid-shaped scolex and sucker; 1 row of rostellar hooks with no. 100; 5–6 rows of sucker hooks; 4–12 eggs in egg pouch	Kassem [55]
Raillietina echinobothrida	–	–	–	–	Nearly round-shaped scolex and sucker; unilateral genital pore opening with posterior location to middle portion of segment margin; 2 rows of hammer-shaped rostellar hooks; 8–12 eggs in egg pouch	Al–Marsomy and Al–Hamadaani [56]
Raillietina saudiae sp. nov.	0.067 ± 0.001 (0.046–0.071) × 0.037 ± 0.001 (0.023–0.041)	0.173 ± 0.01 (0.166–0.189) × 0.112 ± 0.01 (0.094–0.120)	0.018 ± 0.001 (0.017–0.019) × 0.013 ± 0.01 (0.012–0.014)	0.024 ± 0.001 (0.020–0.029) × 0.019 ± 0.001 (0.017–0.023)	2 rows of hammer-shaped rostellar hooks with no. 230–250 (240), 5–6 rows of sucker hooks, testes no. 27–37, unilateral genital pore with anterior location to middle portion of margin, 5–6 eggs in egg pouch	(Present study)

Table 2
Cestoda species used in the phylogenetic analysis of *R. saudiae* sp. nov. specimens obtained in this study for their corresponding ITS2 and ND1 gene regions.

Parasite species	Order/Family	ITS2 gene region				ND1 gene region			
		Host/Host group (Origin)	Accession no.	GC content	Percent identity	Host/Host group (Origin)	Accession no.	GC content	Percent identity
Taenia asiatica	Cyclophyllidea/Taeniidae	*Sus crofa* (Taiwan)	AY606272.1	59.4%	93.0%	–	–	–	–
Taenia pisiformis	Cyclophyllidea/Taeniidae	*Oryctolagus cuniculus* (Taiwan)	JX317675.1	52.0%	93.0%	–	–	–	–
Taenia hydatigena	Cyclophyllidea/Taeniidae	*Canis lupus familiaris* (China)	FJ886761.1	52.6%	93.0%	*Stray dog* (Turkey)	KC876043.1	29.8%	74.0%
Taenia multiceps	Cyclophyllidea/Taeniidae	–	–	–	–	*Capra aegagrus* (*Greece*)	KR604804.1	30.0%	75.0%
Mesocestoides litteratus	Cyclophyllidea/Mesocestoididae	*Felis catus* (Germany)	MH936660.1	57.2%	93.0%	–	–	–	–
Mesocestoides corti	Cyclophyllidea/Mesocestoididae	–	–	–	–	*Mus musculus* (Colorado)	AP017667.1	27.5%	74.0%
Staphylocystis schilleri	Cyclophyllidea/Hymenolepididae	*Sorex palustris* (USA)	KF257896.1	54.1%	93.0%	–	–	–	–
Hymenolepiss nana	Cyclophyllidea/Hymenolepididae	*Rattus rattus* (China)	JF766715.1	51.5%	94.0%	–	–	–	–
Hymenolepis folkertsi	Cyclophyllidea/Hymenolepididae	–	–	–	–	*Peromyscus leucopus* (North America)	KX782315.1	30.3%	75.0%
Hymenolepis diminuta	Cyclophyllidea/Hymenolepididae	*Rattus norvegicus* (Egypt)	MF143799.1	49.0%	93.0%	–	–	–	–
Hymenolepis sp.	Cyclophyllidea/Hymenolepididae	–	–	–	–	*Apodemus sylvaticus* (Turkey)	HM149297.1	28.2%	75.0%
Moniezia expansa	Cyclophyllidea/Anoplocephalidae	*Capricornis crispus* (North Tohoku)	AB367793.1	53.0%	94.0%	*Buffalo calves* (China)	NC036219.1	29.5%	75.0%
Raillietina cesticillus	Cyclophyllidea/Davaineidae	*Gallus Gallus domesticus* (Germany)	KP893422.1	52.0%	95.0%	–	–	–	–
Raillietina echinobothrida	Cyclophyllidea/Davaineidae	*Pseudopodoces humilis* (China)	MH122787.1	50.5%	95.0%	–	–	–	–
Raillietina australis	Cyclophyllidea/Davaineidae	–	–	–	–	*Dromaius novaehollandiae* (Australia)	EU665484.1	30.7%	77.0%
Raillietina tetragona	Cyclophyllidea/Davaineidae	*Pseeudopodoces humilis* (China)	MH122788.1	49.7%	97.0%	–	–	–	–
Raillietina beveridgei	Cyclophyllidea/Davaineidae	*Dromaius novaehollandiae* (Australia)	AY382318.1	50.2%	96.0%	–	–	–	–
Raillietina tunetensis	Cyclophyllidea/Davaineidae	–	–	–	–	*Leptotila verreauxi* (Costa Rica)	EU665487.1	31.6%	78.0%
Raillietina chiltoni	Cyclophyllidea/Davaineidae	*Dromaius novaehollandiae* (Australia)	AY382319.1	48.2%	97.0%	–	–	–	–
Raillietina dromaius	Cyclophyllidea/Davaineidae	*Dromaius novaehollandiae* (Australia)	AY382320.1	48.5%	97.0%	–	–	–	–
Raillietina coreensis	Cyclophyllidea/Davaineidae	–	–	–	–	*Apodemus agarius* (South Korea)	KP171531.1	28.5%	76.0%
Raillietina echinobothrida	Cyclophyllidea/Davaineidae	*Gallus gallus* (India)	JN797628.1	54.8%	95.0%	–	–	–	–
Raillietina sonini	Cyclophyllidea/Davaineidae	–	–	–	–	*Dendrocopos syriacus* (Bulgaria)	EU665490.1	29.5%	76.0%
Raillietina sp.	Cyclophyllidea/Davaineidae	–	–	–	–	*Homo sapiens* (USA)	KT001066.1	31.5%	78.0%
Skrjabinia cesticillus	Cyclophyllidea/Davaineidae	*Gallus gallus* (Australia)	AY382321.1	50.2%	94.0%	–	–	–	–
Parorchites zederi	Cyclophyllidea/Dilepididae	*Aptenodytes forsteri* (Antarctica)	KP893424.1	52.1%	93.0%	–	–	–	–
Pseudanoplocephala crawfordi	Cyclophyllidea/Hymenolepididae	–	–	–	–	*Sus crofa* (China)	KR611041.1	30.3%	74.0%
Drepanidotaenia lanceolata	Cyclophyllidea/Hymenolepididae	–	–	–	–	*Rhine geese* (China)	KR817910.1	30.1%	75.0%
Dipylidium caninum	Cyclophyllidea/Dipylidiidae	–	–	–	–	*Felis catus* (Unknown)	AB732959.1	27.1%	74.0%
Anoplocephala perfoliata	Cyclophyllidea/Anoplocephalidae	*Equus ferus* (Germany)	AJ578153.1	49.4%	93.0%	–	–	–	–
Lineolepis scutigera	Cyclophyllidea/Hymenolepididae	*Sorex tundrensis* (Mountain Altai)	GU299859.1	52.9%	94.0%	–	–	–	–
Paruterina candelabraria	Cyclophyllidea/Paruterinidae	–	–	–	–	*Athene noctua* (China)	NC039533.1	26.7%	74.0%
Arostrilepis tenuicirrosa	Cyclophyllidea/Hymenolepididae	*Myodes glareolus* (Lithuania)	JX121632.1	51.4%	94.0%	–	–	–	–
Diphyllobothrium nihonkaiense	Diphyllobothriidea/ Diphyllobothriidae	–	–	–	–	*Canis lupus* (Canada)	HQ423295.1	32.6%	70.0%

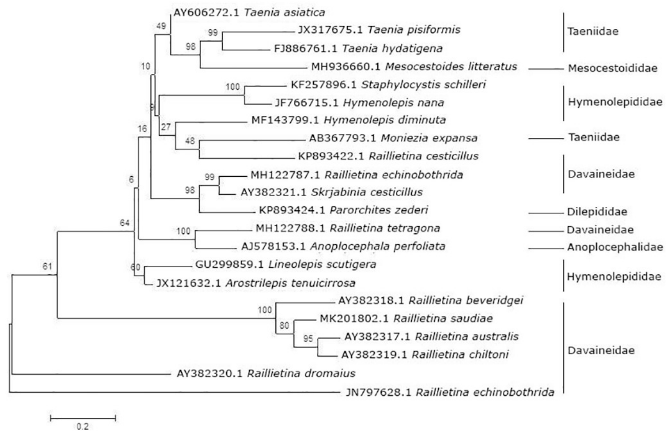

Fig. 4. Neighbour–joining (NJ) and maximum–likelihood (ML) bootstrap consensus tree with 1000 bootstrap replicates of ITS2. The optimal tree with the sum of branch length = 6.61477958 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The analysis involved 22 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were 30 positions in the final dataset. Evolutionary analyses were conducted using MEGA6.

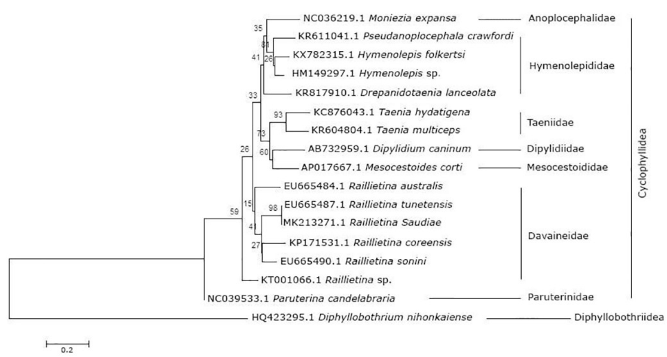

Fig. 5. Neighbour–joining (NJ) and maximum–likelihood (ML) bootstrap consensus tree with 1000 bootstrap replicates of ND1. The optimal tree with the sum of branch length of 4.33453110 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The analysis involved 17 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were 406 positions in the final dataset. Evolutionary analyses were conducted using MEGA6.

shown that the present species is deeply embedded in the genus *Raillietina* with close relationships to the previously described *R. chiltoni*, *R. dromaius*, and *R. beveridgei* as a putative sister taxon.

For the ND1 gene region, highest BLAST scores with lowest divergence values were recorded after comparison between the nucleotide sequence of the present *R. saudiae* sp. nov. and *R. australis* (gb| EU665484.1), *R. tunetensis* (gb| EU665487.1), *R. coreensis* (gb| KP171531.1), *R. sonini* (gb| EU665490.1), and *Raillietina sp.* (gb| KT001066.1). The constructed phylogenetic dendrogram showed 17 nucleotide sequences, divided into two main lineages (Fig. 5). The first lineage included the most closely related families belonging to the order Cyclophylidea, with sequence similarities ranging between 74 and 78%, while the second lineage included species related to the order Diphyllobothriidea. This analysis demonstrated that Paruterinidae is the most basal Cyclophylidea family, forming the sister group to Davaineidae with moderate nodal support. Taeniidae and Hymenolepididae formed a sister group to Anoplocephalidae, Dipylidiidae, and Mesocestoidiae with lower nodal support. This analysis recovered a clade including the monophyletic Davaineidae. Our phylogenetic analysis, incorporating new and existing data investigated the placement of the examined cyclophylidea species within Davaineidae. It was shown that the present species is deeply embedded in the genus *Raillietina* with close relationships to the previously described *R. coreensis* and *R. sonini* as a putative sister taxon. *Diphyllobothrium nihonkaiense* (a representative of the order Diphyllobothriidea) was revealed as the outgroup.

3.4. Chemical analysis

The distribution of elements in the host-parasite system is shown in Table 3. Elements significantly decreased in pigeons infected with cestoda parasite in comparison with non-infected pigeons. The order of metal concentration in the infected pigeon tissues was Zn > Al > Mg > Cu > Cd > Fe > Ni > Mo > Mn > Co > Cr > Pb. The ability of different infected pigeon tissues to accumulate metals was found in the following grading order: ovary > pancreas > kidney > feather > liver > lung > muscle > heart > testes. Zn was the dominant element in all of the evaluated host tissues. In addition, there was high accumulation of Zn in the liver. Large quantities of Zn, Mg, Pb, and Fe accumulate in the ovary, compared with other tissues. In comparison to the other tissues, kidney presented the highest mean concentrations of Al and Mn. In addition, Cu was significantly predominant in the pancreas. Feather accumulated large amounts of Cd, Cr, Ni, Co, and Mo when compared to other pigeon tissues. Also, Al was significantly increased in the lung. Both muscles and testes are the lower organs for metal accumulations. All trace elements presented significantly higher average concentrations in cestoda parasites, than in tissues of host pigeons, in the following order: Al > Zn > Mg > Ni > Mo > Cd > Mn > Cr > Cu > Pb > Co > Fe. Generally, the concentrations of these metals were at risky levels, exceeding the permissible limits recommended by the standards of the US Environmental Protection Agency.

4. Discussion

Only one report has been recorded in Saudi Arabia on the prevalence of gastrointestinal (GI) cestodes belonging to the genus *Raillietina* of the domestic pigeons [52]. *Raillietina* species are cosmopolitan parasites that contribute to nutrient depletion in birds [14]. The present investigation demonstrated a high prevalence (77.78%) of adult *Raillietina* species in small intestines of infected pigeons (*C. livia domestica*). This result was in line with Diakou et al. [57] who reported that the prevalence rate (70.58%) of cestoda fauna of feral pigeons in Thessaloniki off, Northern Greece. However, this result was higher than the data recorded in Nineva and some areas of Erbil and Duhouk provinces (0.66% [58]), Al–Basrah (67.4% [59]), Egypt (34.76% [60], and 30.9% [61]), Zaria (27.1% [62]), Kashmir off India (22.5% [63]), Sistan region of Iran (26.08–28.26% [7]), and São Paulo and Minas Gerais (41.5% [64]). In addition, it was lower than the obtained rate in Bangladesh (100% [65]). The variation in overall prevalence rate could be related to poor hygienic measures, abundance and presence of intermediate hosts (such as ants, cockroaches, beetles, earthworms, and some molluscs), the host species and its feeding habits, climate factors such as temperature, which may influence the presence of intermediate hosts, and geographical factors [7,30,58,59,66–68]. In the present study, the rates of parasitic infections among the male and female pigeons indicates that both sexes are equally exposed to the same risk of acquiring the parasites, which is consistent with Hassouni and Belghyti [69], Adang et al. [62], and Sari et al. [3] whom showed that there was no a usual natural affinity of helminth infection to either sex of pigeons.

Variability in the morphological characteristics upon which the taxonomy of cestodes is based, has received very little attention. Although several reports have described the variation in the morphological characters of cestoda species, few investigators have attempted to determine the frequencies with which these variants occurred in the

Table 3
Effect of parasitic infection on trace element concentrations (mg/g wet weight) in infected pigeon tissues as well as in their parasitic cestoda.

Trace element		Different pigeon tissues									*R. saudiae* sp. nov.
		Liver	Kidney	Heart	Lung	Muscles	Pancreas	Ovary	Testes	Feather	
Cd	Uninfected pigeon	10.65 ± 1.42	8.55 ± 0.28	7.87 ± 0.87	7.98 ± 0.93	3.76 ± 0.67	12.87 ± 2.54	9.75 ± 0.28	3.54 ± 0.87	66.65 ± 7.65	322.52 ± 37.54 [a b]
	Infected pigeon	0.26 ± 0.04 [a]	0.23 ± 0.08 [a]	0.29 ± 0.08 [a]	0.95 ± 0.34 [a]	1.47 ± 0.49 [a]	3.63 ± 1.13 [a]	2.69 ± 1.05 [a]	0.64 ± 0.17 [a]	38.15 ± 6.29 [a]	
Cr	Uninfected pigeon	16.76 ± 3.54	9.76 ± 0.87	8.76 ± 0.96	5.76 ± 0.78	5.04 ± 0.89	8.56 ± 0.92	10.77 ± 1.06	7.55 ± 0.89	21.86 ± 3.87	191.14 ± 39.37 [a b]
	Infected pigeon	1.37 ± 0.03 [a]	1.69 ± 0.04 [a]	0.32 ± 0.07 [a]	0.51 ± 0.08 [a]	0.53 ± 0.19 [a]	2.72 ± 0.10 [a]	3.62 ± 1.13	0.41 ± 0.09 [a]	8.01 ± 2.06 [a]	
Cu	Uninfected pigeon	30.65 ± 4.55	27.87 ± 3.76	16.87 ± 2.87	23.77 ± 3.65	35.87 ± 4.65	50.65 ± 6.75	60.64 ± 8.86	24.86 ± 4.75	9.64 ± 0.89	160.81 ± 13.60 [a b]
	Infected pigeon	12.65 ± 1.21 [a]	9.45 ± 0.84 [a]	4.62 ± 0.59 [a]	4.85 ± 0.39 [a]	13.02 ± 2.06 [a]	23.27 ± 3.68 [a]	18.64 ± 3.26 [a]	4.14 ± 0.79 [a]	0.15 ± 0.03 [a]	
Mn	Uninfected pigeon	40.43 ± 9.86	60.75 ± 7.98	30.75 ± 6.87	35.87 ± 10.98	26.87 ± 4.87	40.76 ± 3.98	22.97 ± 3.65	30.76 ± 3.75	60.74 ± 9.64	247.57 ± 23.23 [a b]
	Infected pigeon	3.78 ± 0.15 [a]	8.77 ± 1.43 [a]	0.06 ± 0.002 [a]	2.10 ± 0.82 [a]	0.15 ± 0.03 [a]	2.52 ± 0.03 [a]	0.064 ± 0.002 [a]	0.40 ± 0.13 [a]	9.43 ± 1.51 [a]	
Pb	Uninfected pigeon	20.87 ± 3.67	19.76 ± 2.76	23.77 ± 3.96	17.87 ± 2.97	30.76 ± 3.65	50.76 ± 6.87	65.85 ± 7.87	37.76 ± 6.87	20.65 ± 5.75	93.59 ± 11.88 [a b]
	Infected pigeon	1.79 ± 0.63 [a]	1.89 ± 0.83 [a]	1.12 ± 0.13 [a]	0.86 ± 0.13 [a]	1.72 ± 0.40 [a]	3.19 ± 0.53 [a]	7.12 ± 1.32 [a]	0.33 ± 0.13 [a]	0.46 ± 0.10 [a]	
Zn	Uninfected pigeon	130.79 ± 20.77	110.07 ± 17.87	69.76 ± 11.84	70.65 ± 10.88	56.98 ± 9.76	89.67 ± 11.76	240.76 ± 25.87	70.75 ± 8.63	24.84 ± 4.85	554.48 ± 62.98 [a b]
	Infected pigeon	86.99 ± 7.29 [a]	48.79 ± 6.19 [a]	16.12 ± 1.27 [a]	25.81 ± 3.24 [a]	25.72 ± 2.61 [a]	46.60 ± 3.98 [a]	117.83 ± 14.56 [a]	15.86 ± 2.01 [a]	0.58 ± 0.21 [a]	
Ni	Uninfected pigeon	30.85 ± 7.74	40.75 ± 9.74	25.87 ± 10.76	60.76 ± 9.57	63.87 ± 8.67	80.65 ± 7.09	79.78 ± 6.75	27.09 ± 3.76	78.02 ± 9.87	348.83 ± 35.01 [a b]
	Infected pigeon	1.02 ± 0.13 [a]	3.47 ± 0.71 [a]	0.77 ± 0.08 [a]	1.31 ± 0.12 [a]	2.79 ± 0.22 [a]	4.71 ± 0.51 [a]	5.38 ± 0.35 [a]	1.11 ± 0.08 [a]	23.86 ± 13.72 [a]	
Fe	Uninfected pigeon	19.01 ± 1.78	18.98 ± 1.87	19.74 ± 1.09	29.63 ± 4.84	12.73 ± 0.87	10.73 ± 0.98	38.74 ± 2.73	12.84 ± 1.97	14.73 ± 0.91	66.85 ± 12.72 [a b]
	Infected pigeon	4.41 ± 0.02 [a]	4.85 ± 0.04 [a]	4.15 ± 0.97 [a]	9.42 ± 1.79 [a]	1.53 ± 0.58 [a]	2.58 ± 0.95 [a]	15.10 ± 3.36 [a]	0.98 ± 0.30 [a]	3.63 ± 1.16 [a]	
Mg	Uninfected pigeon	37.98 ± 4.98	69.87 ± 7.90	89.87 ± 6.75	38.76 ± 5.86	58.87 ± 9.01	65.87 ± 9.56	70.75 ± 11.90	49.76 ± 3.76	50.65 ± 8.67	478.99 ± 62.76 [a b]
	Infected pigeon	6.49 ± 1.14 [a]	12.27 ± 2.59 [a]	22.96 ± 2.04 [a]	6.73 ± 1.66 [a]	10.04 ± 1.48 [a]	21.39 ± 3.82 [a]	53.91 ± 8.12 [a]	8.70 ± 1.89 [a]	19.10 ± 2.17 [a]	
Al	Uninfected pigeon	84.65 ± 8.89	110.87 ± 17.76	76.74 ± 7.90	130.65 ± 23.67	97.09 ± 11.54	103.65 ± 15.82	109.33 ± 11.60	64.87 ± 6.64	56.23 ± 6.75	656.91 ± 42.36 [a b]
	Infected pigeon	19.67 ± 4.61 [a]	76.99 ± 8.74 [a]	18.24 ± 3.55 [a]	63.55 ± 9.58 [a]	30.93 ± 7.20 [a]	60.32 ± 6.10 [a]	65.57 ± 8.89 [a]	19.47 ± 4.23 [a]	5.52 ± 1.74 [a]	
Co	Uninfected pigeon	20.76 ± 2.12	32.90 ± 4.98	19.76 ± 2.97	56.32 ± 6.98	45.98 ± 2.76	34.67 ± 2.91	29.53 ± 1.07	14.94 ± 0.92	31.07 ± 2.92	79.80 ± 6.69 [a b]
	Infected pigeon	0.23 ± 0.09 [a]	0.67 ± 0.05 [a]	0.21 ± 0.04 [a]	4.28 ± 3.85 [a]	1.58 ± 0.59 [a]	1.29 ± 0.77 [a]	2.21 ± 0.87 [a]	0.45 ± 0.22 [a]	13.09 ± 7.58 [a]	
Mo	Uninfected pigeon	40.76 ± 8.83	54.92 ± 7.08	30.06 ± 2.09	40.86 ± 3.86	27.05 ± 3.86	59.01 ± 4.90	67.98 ± 6.93	49.92 ± 5.89	80.55 ± 4.98	335.70 ± 31.90 [a b]
	Infected pigeon	1.98 ± 0.04 [a]	2.41 ± 0.08 [a]	0.31 ± 0.09 [a]	1.10 ± 0.59 [a]	0.37 ± 0.17 [a]	3.30 ± 1.38 [a]	4.64 ± 1.17 [a]	0.94 ± 0.31 [a]	10.57 ± 1.69 [a]	

Values are means ± SE. [a] significance at $P \le 0.05$ against control group, [b] significant at $P \le 0.05$ against infected group.

species exhibiting them [12,16,29,30,70]. In the family *Davaineidae*, where some attempts have been made, the analyses have been conducted using specimens collected from naturally infected hosts. Most variant characters known in cestoda species within the family *Davaineidae* are related to: (i) shape and size of scolex, (ii) shape, size of suckers, and number of its hooks, (iii) number, shape, size and situation of rostellar hooks, (iv) number, size, shape and relative position of the testes, (v) shape of ovary and vitellarium, (vi) position and number of the genital pores per segment, and (vii) number and size of eggs within an egg capsule in gravid proglottids. These features constitute important taxonomic characters in this family and consequently, they have been the most satisfactorily described internal structures. In the present study, the recovered cestoda parasite was compared with those from different regions and showed that it is similar to all comparable species in terms of the generic characteristic features in addition to inhabiting the same host species. Also, it closely resembled *R. canabia* Magzoub, Kasim, Shawa [52] and *R. zahratis* Magzoub, Kasim, Shawa [52] in having the same host locality in Saudi Arabia. It can be separated from all comparable species by smaller body size compared with that of *R. crassula*, *R. clerici*, *R. torquata*, *R. nagpurensis* Moghe [44], and *R. horkei* Joyeux and Houdemer [45], and by larger body size compared to that of other comparable species. In addition, it can be characterised by larger size of scolex compared to that of *R. bonini* Megnin [38], *R. torquata*, *R. weissi* Joyeux and Baer [49], *R. tokyoensis*, *R. zahratis*, *R. tetragone* Kassem [55], and *R. echinobothrida*, and smaller size of scolex compared to that of other comparable species.

Intestinal cestodes are frequently identified on the basis of morphological features, their transmission patterns, or their pathological effects on their hosts [71]. However, these criteria are often insufficient for specific identification [72]. The possibility of using molecular approaches for species identification has contributed to increased knowledge of the genus *Raillietina*; however, the phylogenetic relationships of *Raillietina* species in domestic pigeons from Saudi Arabia had not been studied before. Wolf et al. [73] and Ramnath et al. [17] assessed the validity of the ITS2 gene region in discriminating closely related *Raillietina* species and recommended that further validation needs to be well established to exploit the ITS2 and other markers for robust molecular characterization of these avian parasites. Recently, the ribosomal DNA and mitochondrial genes have been widely used to identify and study phylogenetic relationships among species because of their fast evolutionary rates at the species and genus levels [73–78].

The cestoda group of parasites belonging to the orders Cyclophyllidea, Diphyllobothriidea, Caryophyllidea and Trypanorhyncha has been studied based on morphological, ultrastructural and ontogenetic characters [79–83]. In the present study, ITS2 and ND1 gene regions were investigated in the context of phylogenetic relationships of the recovered *Raillietina* species. The phylogenetic trees derived from both ITS2 and ND1 gene regions showed that each *Raillietina* species was separated in correlation with the morphological characters and their definitive host. This result is consistent with O′ Callaghan et al. [84], Littlewood et al. [85], and Butboonchoo and Wongsawad [86] whom stated that *R. echinobothrida* and *R. tetragona* are more related to each other than to the other *Raillietina* species because their definitive host is domestic chickens whereas the other *Raillietina* species use other birds as their definitive host. In addition, Butboonchoo et al. [87] reported that the phylogenetic relationships obtained using the ITS2 gene region and the gravid proglottid characters could separate *Raillietina* species into two groups. In the present study, the phylogenetic relationships obtained using the ND1 sequence showed a phylogenetic tree different from that based on the ITS2 gene region as all *Raillietina* species were grouped according to the unilateral with alternating position of the genital pore and the egg numbers in each egg capsule.

BLAST hits on the query sequences showed that sequences of the current *Raillietina* species denoted homology with maximum identity up to 92.0% for ITS2 and 72.0% for ND1. In addition, the query gene sequences of *R. saudiae* sp. nov. were aligned and embedded within the Davaineidae group with other species of *Raillietina* and separated from Hymenolepididae and Taeniidae as a relative group and from Diphyllobothridae as an out-group. These results are similar to the results obtained by Jones and Beveridge [88], Khalil et al. [16], and Ramnath et al. [17] who showed *Raillietina* spp. neighbouring the position with other studied species of the order Cyclophyllidea against the outgroup Order Diphyllobothriidea according to the degree of maximum identity with lower divergence gaps. In addition, Guo [89] used the Maximum Likelihood Method to construct the phylogenetic tree and representatives of Diphyllobothriidea and Cyclophyllidea (Anoplocephalidae, Hymenolepididae, and Taeniidae) with strongly supported independent clades. Hymenolepididae exhibited a sister-group relationship with Anoplocephalidae, consistent with previous studies based on 18S rDNA [90–94], 12S rDNA [95], and COX genes [96]. This is also in line with the cladistics analyses based on morphological characters by Hoberg et al. [97]. In addition, our data corroborate Khalil et al. [98] who demonstrated the arrangement of mesocestodides into Cyclophyllidea with narrow relationship between it and Taeniidae, Hymenolepididae and Anoplocephalidae. The present results revealed that the ITS2 and ND1 gene regions recorded from *Raillietina* species appeared to be monophyletic. This is consistent with Khalil et al. [16] who investigated the monophyly of *Raillietina* species based on the query sequences of the ITS2 and ND1 gene regions with bootstrapping of both phylogenetic trees indicating significant supports for species grouping.

The present study showed a close phylogenetic relationship between *Paruterina candelabraria* and *Raillietina* species, which confirmed the hypothesis of Swiderski and Tkach [99], Hoberg et al. [97], Foronda et al. [6], and Dimitrova et al. [100] who used the homology of the paruterine organ with the egg capsules, a characteristic of *Raillietina* species, to justify this relationship. In addition, the current study supports the taxonomic position of the recovered davaineid species with a unique genetic sequence that is deeply embedded in a genus that includes *R. beveridgei, R. australis, R. chiltoni, R. coreensis, R. tunetensis,* and *R. sonini* as a putative sister taxon.

Certain organisms can be used to obtain information about the chemical state of their environment their presence or absence [28,101–104]. Recently, evaluation of environmental pollution has considered the ability of parasites to concentrate inorganic elements, particularly heavy metals, at much higher levels compared to free-–living organisms [105]. From a public health viewpoint, it is necessary to determine heavy metal concentrations in different animal species used for human consumption [106]. In the present study, pigeons infected with cestodes had lower concentrations of heavy metals in their tissues than those in uninfected pigeons. In addition, the metal concentrations of cestoda parasites was compared to those of different organs of its host. It accumulates heavy metals 4 orders of magnitude higher than those in their pigeon hosts. In this context, Sures et al. [107] and Barus et al. [108] concluded that some parasites of the terrestrial hosts accumulate toxic elements more efficiently than their hosts and intestinal parasites can accumulate more metals compared to parasites inhabiting the body cavity [109]. Torres et al. [24] proposed the model *R. micracantha/C. livia* from the city of Santa Cruz de Tenerife (Canary Islands, Spain) as a promising bioindicator in evaluating environmental pollution of heavy metals particularly Pb and Mn.

The current investigation showed that liver and kidney were the most important organs for assessing metal accumulation, as the levels of heavy metals in them reflect the storage of metals in the host body. These results are consistent with those reported by Florence et al. [102], Muir et al. [103], Yilmaz et al. [110], and Forstner and Wittmann [111]. The ovary of infected pigeons has the highest level of metal accumulation, probably by the presence of higher quantities of Pb.

For essential elements, relatively higher concentrations of Zn have often been detected without any poisonous effect on the health of the organisms. Moreover, interaction of Zn with particular toxic elements as Cd and Pb may even reduce their toxicity [112]. The current investigation demonstrated the distribution of Zn in different organs of infected pigeons, which is in agreement with Pourang [113] who studied the accumulation of different heavy metals in *Esox lucius* and *Carassius auratus* from Anzali wetland, Iran. On the other hand, a higher concentration of Cu is usually toxic [114]. Bíreš et al. [115] demonstrated that Zn can inhibit the accumulation of Cu in animal tissues and, hence, it provides certain protection against toxic effects of Cu. The most affected elements were Fe, Cu, Zn, Mn, and Co, which showed lowers concentrations in the host muscles. This suggested the essential pathogenic effect of infection by *Anguillicola crassus* on the quality and usable biomass of the host [116]. In the present study, the highest mean concentrations of Cd, Cr, Ni, Co, and Mo were found in pigeon feathers compared with soft tissues. This is consistent with Ek et al. [117] who reported that metal element concentration in the feather can generally be ascribed to both exogenous contamination and mobilization from internal trace element metabolisation.

5. Conclusion

This field study provides new insights for rapid identification, systematics, and phylogenetic analysis of davaineids infecting pigeons. In addition, it demonstrates that ITSs and ND1 gene regions of *R. saudiae* sp. nov. yield a unique sequence that confirms its taxonomic position within the family Davaineidae. Our results also demonstrate that the domestic pigeons *C. livia domestica* ought to be considered a possible natural reservoir of different species of cestoda parasites. Further investigation should focus on analysis of different genes to clarify the phylogenetic relationships of Davaineidae.

Conflict of interest

The authors have indicated that they have no conflict of interest regarding the content of this article.

Compliance with ethical standards

The study was approved by the ethical committee of the parasitology group at the College of Science, King Saud University for the project RG-002.

Acknowledgements

Authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through research group no (RG-002).

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