Original article

Morphological and molecular approaches of the nematode parasite *Desportesius invaginatus* (Acuariidae) infecting the cattle egret *Bubulcus ibis* (Ardeidae)

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ARTICLE INFO

ABSTRACT

Investigations on the parasites that infect migrating birds worldwide are ongoing. To identify the parasite species that infect ardeids, morphological and morphometric characteristics were used. A total of 20 *Bubulcus ibis* (Ardeidae) specimens were collected from the agricultural lands belonging to the Faculty of Agricultural at Cairo University, which were then examined for nematode parasites. Only one acuariid species, belonging to the Acuariidae family, has been identified, with a prevalence rate of 50 % (10/20) among infected egrets. The *Desportesius* species isolated from the gizzard of the egret host is morphologically and morphometrically compatible with *Desportesius invaginatus*, which was previously identified from several Ciconiiformes hosts of *Bubulcus ibis* (Egypt, India, and Taiwan), *Egretta garzetta* (France), and *Egretta rufescens* (USA). Additionally, utilizing the partial small subunit ribosomal RNA (*18S rRNA*) gene sequence, maximum parsimony based on the Tamura-Nei model was used to infer the phylogeny of the recovered *Desportesius* species. The query sequences revealed 99.37 % identity for the *18S* (MW358651.1) of the previously mentioned *D. invaginatus*. In addition to clarifying several morphological features of *D. invaginatus*, this study also provided new DNA data for this species.

1. Introduction

Small nematodes known as acuarioids are frequently found inside the esophagus, proventriculus, and gizzard of birds, notable waders from the Scolopacidae and Charadriidae families. The cordons, ptilina, or serrated shields seen on the cephalic structures of members of the superfamily are distinctive features (Chabaud, 1975). Species with cordons are members of the Acuariinae subfamily (there is only the single-family Acuariidae in the superfamily).

Chabaud and Campana (1949) designated the parasitic helminths that are mostly found in fish-eating birds of the order Ciconiiformes as belonging to the genus *Desportesius* (Nematoda, Acuariidae). Although many academics now acknowledge it as a distinct genus, it was once thought to be a subgenus of *Synhimantus* (Chabaud and Petter, 1959; Skryabin et al., 1965; Chabaud, 1975; Wong and Anderson, 1986; Anderson et al., 1996). *Desportesius* is a genus that has about eight species, however, because of how similar they are, it can be difficult to classify them (Chabaud and Campana, 1949; Wong and Anderson, 1986; Abou Shafeey, 2019).

The most common acuariid parasitic nematode found in Egypt's egret gizzards is *Desportesius invaginatus* Skrjabin, Sobolev et Ivaschkin, 1965 which inhabits the gizzard of egrets in Egypt. The morphology of *Desportesius invaginatus*' morphology has been researched by several authors (Skrjabin, 1917; Skryabin et al., 1965; Baruš et al., 1978; Wong and Anderson, 1986; Smogorzhevskaya, 1990; Varjabedian, 2006; Wheeb et al., 2015). The previous redescription only provided information on a few

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Peer review under responsibility of King Saud University.

characters and considered the metrical data and its variance (Mutafchiev and Georgiev, 2009).

The primary method for identifying species has been morphological observation, which required extensive training and was a time-consuming process (Dkhil et al., 2022). As a defense mechanism, molecular methods of nematode identification offer precise and different diagnostic techniques (Nega, 2014). The primary taxonomic identifier for species identification has been the DNA sequence of the target regions (Al-Hoshani et al., 2021). Analysis of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene and the nuclear 18S and 28S ribosomal RNA genes served as the basis for the genetic identification of acuariid parasites (Choi et al., 2014; Ebner et al., 2017; Kim et al., 2018; Ivanova et al., 2018; Mutafchiev et al., 2020). The nuclear ribosomal DNA 18S rRNA gene is very repetitive, comprises variable regions flanked by more conserved parts, and has only recently been utilized to research inter- and intraspecific connections, not to identify *Desportesius* specimens (Lee et al., 2021). Even though *Desportesius* specimens' genetic divergence implies that they are conspecific with *Synhimantus* species (Mutafchiev and Georgiev, 2009).

To learn more about the morphology and phylogeny of the acuariid parasites infecting the cattle egret in Egypt, this study was designed.

2. Materials and methods

2.1. Experimental animals

Twenty cattle egrets, *Bubulcus ibis* (Family Ardeidae), were randomly selected from the agricultural lands owned by the Faculty of Agricultural at Cairo University and transported using special boxes to the Parasitology Laboratory in the Department of Zoology, Faculty of Science, Cairo University, Egypt; for further examination. According to Goodman et al. (1989), collected samples were taxonomically recognized.

3. Parasitological examination

3.1. Light microscopic (LM) examination

Within 8 to 24 hrs of collection, each chosen bird was sacrificed by receiving an intraperitoneal injection of a dilute solution of Nembutal (sodium pentobarbital). Each specimen's alimentary canal was dissected, separated into segments, and inspected using a stereo-dissecting microscope (Nikon SMZ18, NIS ELEMENTS software) to check for intestinal parasites. Using a brush or tiny pipettes, the collected intestinal parasites were transferred to a

Fig. 1. Light photomicrograph of *Desportesius invaginatus* showing different body parts. (A) Whole mount preparation. (B-N) High magnifications for different body parts: (B-E) Anterior extremity of the body. (F) Deirids. (G) The esophagus is followed by the intestinal diverticula (arrow) and intestine. (H) The middle part of the body shows testes. (I-K) The posterior extremity of the female worm. (L–N) The posterior extremity of the male worm with the cloacal opening (arrow). Note: PL, pseudolabia; BC, buccal capsule; CO, cordons; TA, transverse annulations; DR, deirids; SPU, spinules; ES, esophagus; TE, testes; IN, intestine; LA, lateral alae; T, tail; P, papillae; MO, mouth opening; PCP, precloacal papillae; POCP, postcloacal papillae; AN, anal opening; SP, spicules.

clean saline solution and repeatedly washed to remove any mucus or debris that was typically adhering to their body surface. For further investigation and identification, the helminth parasites were fixed in 70 % ethanol. For each egret, the sites and the numbers of each parasite species were noted. According to Bush et al. (1997), the parasitological term of the prevalence was estimated.

3.2. Morphology and morphometry

To prepare the worms for whole mounts (n = 5), they were first fixed, stained with Semichon's acetocarmine, dried using a graduated ethanol series, cleared in clove oil, and mounted with Canada balsam in permanent preparations. The parasitic nematode specimens (n = 5) were preserved in pure glycerine as semi-permanent mounts for examination (Ryss, 2003). Using a Leica DM 2500 microscope (NIS ELEMENTS software, version 3.8), the mounted specimens and the pertinent structural features were studied, documented, and photographed at different magnifications. Using ImageJ 1.53e software (Wayne Rasband and contributors, National Institute of Health, USA), measurements were collected from digitalized drawings and represented in millimeters (mm).

3.3. Scanning electron microscopic (SEM) examination

The specimens (n = 10) were fixed at 3 % glutaraldehyde, rinsed in sodium cacodylate buffer, dehydrated in a graded sequence of ethanol, and infiltrated with amyl acetate (Al-Hoshani et al., 2021). They were processed in a critical point dryer "LEICA, EM CPD300" with Freon 13 after passing through an ascending series of Genesolv D, then coated with gold–palladium using an auto fine coater (JEOL, JEC-3000FC), before the examination using an Etec Autoscan at 10-kV JEOL-SEM (JSM-6060LV) in the Electron Microscopy Unit at the Faculty of Science, Ain Sham University.
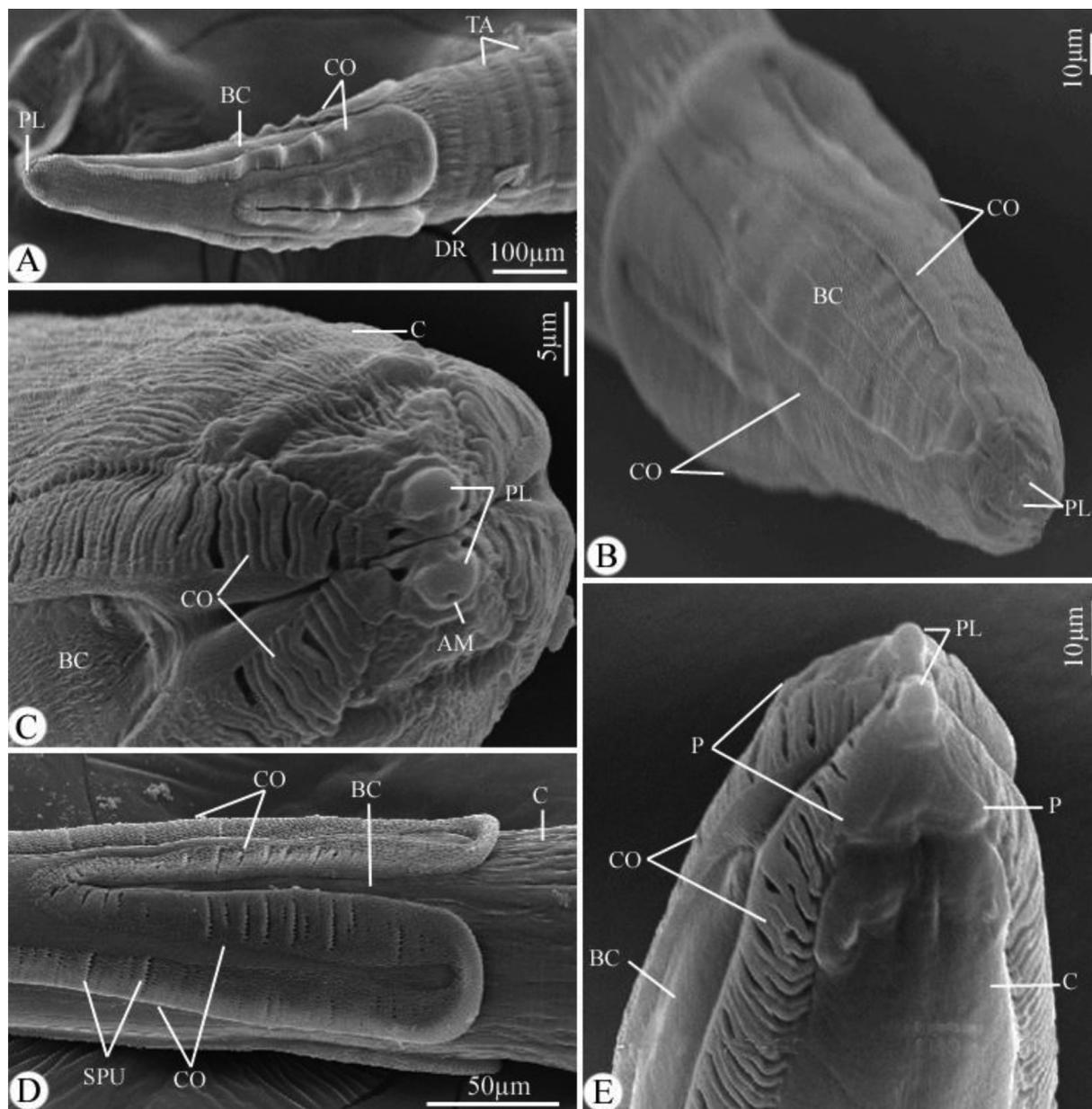

Fig. 2. Scanning electron micrographs of *Desportesius invaginatus* showing different parts in the anterior extremity of the body. Note: AM, amphid; PL, pseudolabia; BC, buccal capsule; CO, cordons; TA, transverse annulations; DR, deirids; C, cuticle; SPU, spinules.

3.4. Type material

Permanent slides and the preserved nematode samples were placed in the collection of parasitology of the Department of Zoology at College of Science, King Saud University, Riyadh, Saudi Arabia.

4. Molecular analysis

4.1. Extraction of the genomic DNA

Using a DNeasy tissue kit© (Qiagen, Hilden, Germany), genomic DNA was extracted from ethanol-preserved samples (n = 10) by following the manufacturer's instructions. Genomic DNA was utilized for the polymerase chain reaction (PCR) and its concentration and purity were measured using a NanoDrop ND-1000 spectropho-

tometer (Thermo Fischer Scientific, Inc., Wilmington, DE, USA) (Al-Hoshani et al., 2021).

4.2. Amplification via polymerase chain reaction (PCR)

Through the PCR, a partial nuclear small subunit ribosomal RNA (18S rRNA) gene was targeted and amplified in this study. A total volume of 50 µl was used for all PCR reactions, which includes 5 µl of 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. Primers used for the targeted gene were 5′-CGT ACC GGC GAC GTA TCT AT-3′ (forward) and 5′-AGG TGA GTT TTC CCG TGT TG-3′ (reverse) as mentioned by Lee et al. (2021). The PCR thermocycling profile included initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 *sec*, annealing at 60 °C for 30 *sec*, and extension at 72 °C
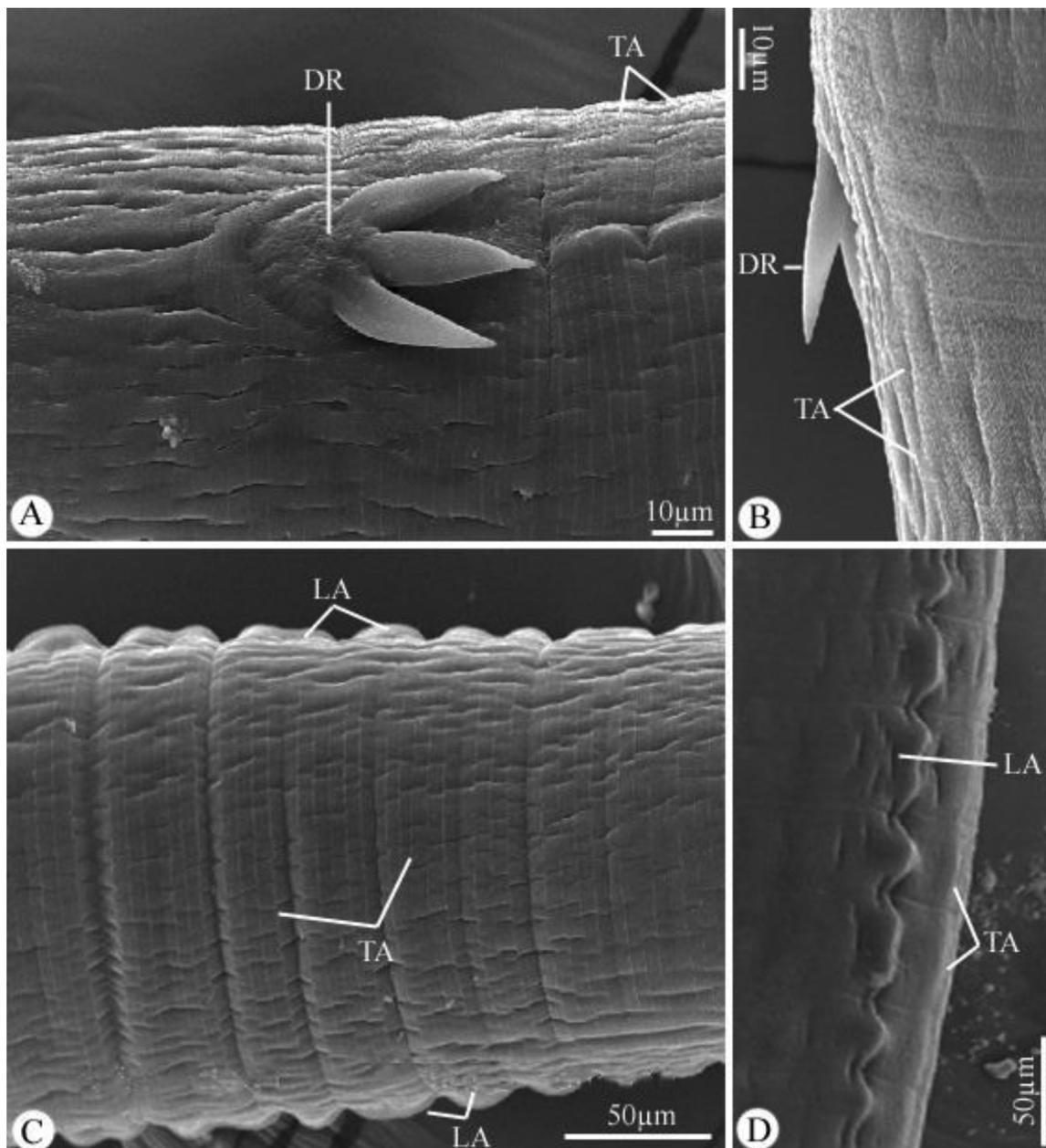

Fig. 3. Scanning electron micrographs of *Desportesius invaginatus* showing different parts in the middle region of the body. Note: DR, deirids; TA, transverse annulations; LA, lateral alae.

for 1 min. The post-PCR extension was then completed for 10 min at 72 °C. On 1.5 % *w/v* agarose gel in 1 × Tris-acetate–EDTA (TAE) stained with SYBR green, the amplicons were examined, and the results were seen with a UV *trans*-illuminator. Following the manufacturer's instructions, PCR products of the anticipated size were gel-extracted and purified using the GeneJET™ PCR Purification Kit [Thermo (Fermentas)] (Al-Quraishy et al., 2020).

4.3. Sequencing and phylogenetic analysis

After sequencing procedures using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA), PCR amplicons were submitted to automated sequencing utilizing an automated DNA Sequencer (ABI-PRISM 310). Using ABI Editview (Perkin-Elmer), each sequence was manually modified for accuracy. Using BioEdit 7.0.1 (Hall, 1999), a partial 18S rRNA gene region was aligned with those in the National Center for Biotechnology Information (NCBI) database. Phylogenetic trees were inferred using Molecular Evolutionary Genetics Analysis (MEGA 7.0) software (Kumar et al., 2016) and Maximum Likelihood (ML) analysis. The bootstrap approach with 1000 replicates was used to evaluate the ML tree. With branch lengths in the same units as the evolutionary distances used to estimate the phylogenetic trees that were rendered to scale.

5. Results

The investigated cattle egret, *Bubulcus ibis*, has nematode parasites that naturally present in ten out of twenty (50 %) specimens, primarily in the gizzard. This parasitic species was identified as *Desportesius invaginatus*. Each parasitized egret has an infection intensity that is between 5 and 10 (mean of 7).

5.1. Morphological studies

The worm has a cylindrical, white body (Fig. 1A). Two conical pseudolabia containing two amphids and a pair of cephalic papillae encircled the mouth opening (Figs. 1A-D, and 2A-C). It had a thin, striated cuticle (Fig. 1G, H). The buccal capsule has the appearance of a long, thin funnel (Fig. 1A-C). Before becoming anastomosed close to the nerve ring, cordons that began at the dorso-ventral sides of the mouth opening extended posteriorly and repeatedly (Figs. 1A-E, and 2A-E). Small spines were given for the cordons. The cordons were laterally followed by a pair of tricuspid deirids (Figs. 1F, and 3A, B). There were lateral alae that were present and extended to the tail's tip behind the deirids (Fig. 1A, I, L, and 3C, D). The cylindrical esophagus has both a muscular and a glandular part (Fig. 1A, B).

5.2. Description of the female worm

The body's dimensions were 9.89–12.88 (11.87) long and 0.30–0.39 (0.37) wide. Cordons were 0.278–0.377 (0.342) long. The length of the buccal cavity was 0.221–0.278 (0.245). The muscular portion of the esophagus measured 0.60–1.28 (1.09) long, while the glandular portion was 2.36–3.01 (2.98) long. The nerve ring and excretory pore were situated at 0.167–0.198 (0.170) and 0.204–0.238 (0.228) from the anterior end, respectively. The tricuspid deirids, which were extremely large, were located 0.432–0.711 (0.667) from the body's anterior end and were 0.047–0.060 (0.056) long. The distance between the vulva and the posterior extremity was 0.051–0.069 (0.061). The tail was conical and had a structure in the form of a rounded knob (Figs. 1I-K, and 4C).
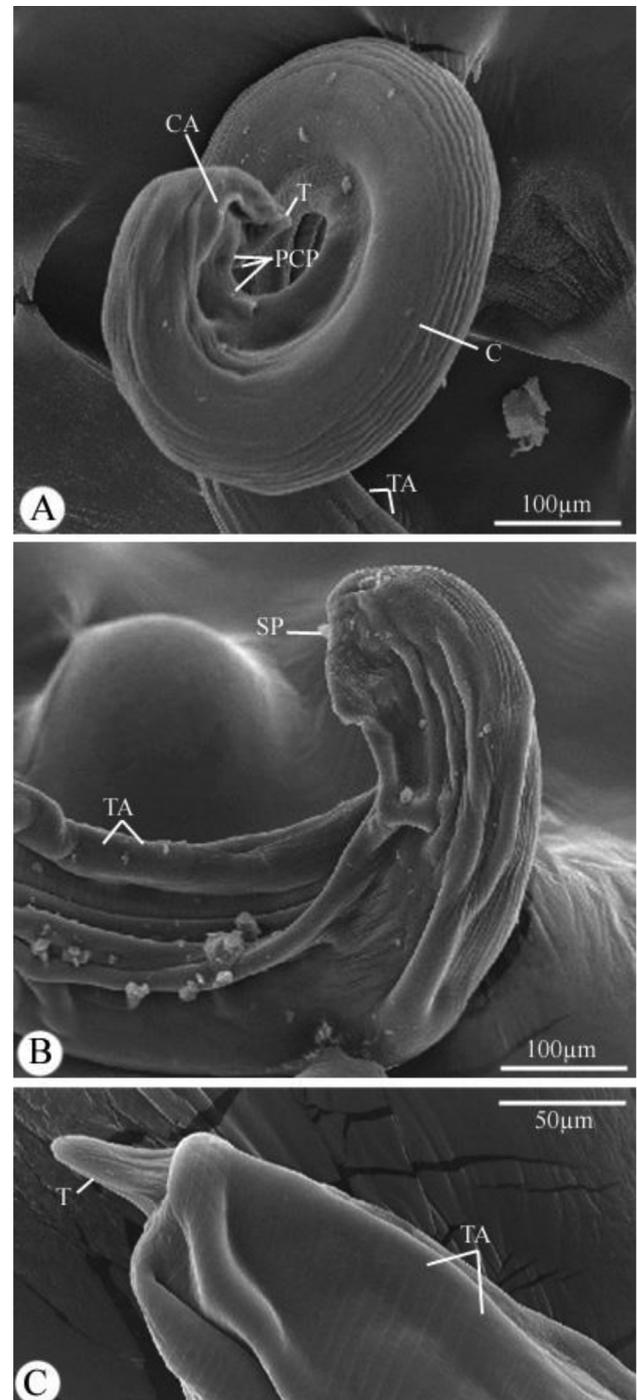
Fig. 4. Scanning electron micrographs of *Desportesius invaginatus* showing different parts in the posterior extremity of the male (A,B) and female (C) body. Note: CA, cervical alae; T, tail; C, cuticle; TA, transverse annulations; SP, spicule.

5.3. Description of the male worm

The body's dimensions were 6.98–8.23 (7.60) long and 0.189–0.201 (0.199) wide. Cordons were 0.234–0.302 (0.298) long. The buccal capsule had a length of 0.199–0.243 (0.220). The muscular portion of the esophagus measured 0.211–0.821 (0.786) long, while the glandular portion was 1.29–2.89 (2.10) long. The nerve ring and excretory pore were situated at 0.147–0.169 (0.158) and 0.201–0.288 (0.225) from the body's anterior end, respectively. Large tricuspid deirids were situated at 0.290–0.632 (0.576) from the body's anterior end and were 0.029–0.039 (0.035) long. The

caudal portion was spirally coiled, and the caudal alae were inflated and vesicular in appearance (Figs. 1L-N, and 4A). Nine pairs of caudal papillae were observed on the posterior extremity, including four pairs of precloacal and five pairs of postcloacal papillae (Fig. 1L, M). Two unequal spicules were present (Figs. 1L, N, and 4B), the right spicule measured 0.690–0.803 (0.729) long, and the left spicule measured 0.401–0.520 (0.492) long.

5.4. Molecular phylogenetic analysis

The partial 18S rRNA sequence of the studied nematode species was 630 bp and included 50.6 % GC content [A(24.6 % 155) | C (21.75 % 137) | G(28.89 % 182) | T(24.76 % 156)] and given the accession number ON564731.1 in GenBank. Nucleotide sequencing data from 26 taxa were aligned across 621 positions using the ML method to construct a phylogenetic dendrogram that represented one class of Chromadorea (Table 1). The average distance between all specimen sequences was 0.030 overall. The identity of the genus *Desportesius* was verified by pairwise comparison with the GenBank 18S rRNA gene data collection (Table 1, and Fig. 5).

Taxa from the following five superfamilies were included in the phylogenetic analysis: Acuarioidea (represented by the family Acuariidae), Habronematoidea (represented by two families Tetrameridae and Habronematidae), Physalopteroidea (represented by family Physalopteridae), Spiruroidea (represented by three families Cystidicolidae, Rhabdochonidae, and Spirocercidae), and Filarioidea (represented by family Setariidae). Different ranges of identities exist for species within the Acuarioidea clade that range from 99.37 to 95.58 %, 98.10–96.35 % for Habronematoidea, 97.78–95.40 % for Physalopteroidea, 97.78–95.29 % for Spiruroidea, and 95.05 % for Filarioidea (Table 1).

A well-resolved separate clade containing acuariids and the recovered nematode species, especially those from the Acuariidae family, was visible on the phylogenetic dendrogram (Fig. 6). The recovered species exhibit high sequence identities for genera within the Acuariidae, with taxa of the *Desportesius* having a 99.37 % identity, *Synhimantus* having a 98.57–98.25 % identity, *Echinuria* having a 95.58 % identity, and *Stegophorus* having a 97.74 % identity. The sequence of the present species clustered with the

Desportesius invaginatus (MW358651.1) sequences from earlier species in the same clade with a strong support value (99).

6. Discussion

Chabaud (1954) categorized the members of the family Acuariidae according to the structure of cordons, and the subfamily Acuariinae consists of 25 genera. These taxa may be identified by the location of the vulva, the monodelphy, the posteriorly enlarged cordons, and the males' inflated, vesicular caudal alae (Chabaud, 1975). The genus *Synhimantus* was divided into three subgenera by Chabaud and Petter (1959), namely *Desportesius*, which has cordons that are anastomosed and posteriorly enlarged, and the vulva is located close to the posterior end; *Synhimantus*, which has cordons that are anastomosed and the vulva is located in the middle of the body; and *Dispharynx*, which has cordons that non-anastomosed and the vulva lied not at the posterior end. The recovered nematode species in this study has the key characteristics of the former subgenus, making it possible to identify it as one *Desportesius* species.

Various ciconiform birds (ardeid birds, primarily herons, bitterns, and egrets) harbor nematodes of the genus *Desportesius* in their gizzard as common and generalist parasites (Chabaud and Campana, 1949; Mawson, 1982; Wong and Anderson, 1986; Anderson et al., 1996), but little is known about their pathogenic effects on the hosts (Tomás et al., 2016). In the current investigation, this *Desportesius* species were found in the gizzard of 50 % of the *Bubulcus ibis* that was examined. This infection rate was lower than that reported by Abou-Shafey (2012), who found *D. invaginatus* in *Ardeola ibis ibis* at an infection rate of 83.33 %, and Wheeb et al. (2015), who found the same parasite species in the cattle egret at an infection rate of 71.42 %.

Desportesius is made up of eight species that are widely known: *D. invaginatus*, *D. brevicaudatus*, *D. bubulcusi*, *D. equispiculatus*, *D. longevaginatus*, *D. orientalis*, *D. sagittatus*, and *D. triaenucha*. The recovered *Desportesius* species was in good agreement with the description and characterization of the previously observed *D. invaginatus* based on both morphological and morphometric features, including the body length, the margins of the tips of the two recurrent cordons are anastomosed on both ventral and dorsal surfaces with the other cordon, length of the muscular and glandular

Table 1
GenBank accession numbers for 18S rRNA sequences used in ML analysis.

Superfamily	Family	Species	Accession No.	% identity
Acuarioidea	Acuariidae	*Desportesius invaginatus*	MW358651.1	99.37
	Acuariidae	*Synhimantus hamatus*	EU004819.1	98.57
	Acuariidae	*Synhimantus laticeps*	EU004818.1	98.41
	Acuariidae	*Synhimantus* cf. *laticeps*	KP861914.1	98.25
	Acuariidae	*Echinuria borealis*	EF180064.1	95.58
	Acuariidae	*Stegophorus macronectes*	HE793715.1	97.74
Habronematoidea	Tetrameridae	*Crassicauda magna*	MT496895.1	98.10
	Habronematidae	*Cyrnea leptoptera*	EU004815.1	96.35
Physaopteroidea	Physalopteridae	*Proleptus* sp.	JF934733.1	97.78
	Physalopteridae	*Heliconema longissimum*	JF803949.1	97.46
	Physalopteridae	*Physaloptera apivori*	EU004817.1	95.40
	Physalopteridae	*Turgida torresi*	EF180069.1	95.40
Spiruroidea	Cystidicolidae	*Cystidicola farionis*	MG594291.1	97.62
	Cystidicolidae	*Comephoronema werestschagini*	MN294781.1	97.78
	Cystidicolidae	*Ascarophis arctica*	DQ094172.1	97.78
	Cystidicolidae	*Neoascarophis longispicula*	JF803921.1	97.62
	Cystidicolidae	*Capillospirura ovotrichuria*	MN294783.1	96.83
	Cystidicolidae	*Cystidicoloides vaucheri*	KY558630.1	96.67
	Cystidicolidae	*Metabronema magnum*	JF803918.1	96.67
	Cystidicolidae	*Salmonema ephemeridarum*	JF803927.1	96.19
	Rhabdochonidae	*Rhabdochona mazeedi*	JF803936.1	96.51
	Rhabdochonidae	*Spinitectus gracilis*	MG594297.1	95.29
	Spirocercidae	*Mastophorus muris*	MG818763.2	96.19
Filarioidea	Setariidae	*Setaria digitata*	DQ094175.1	95.08
	Onchocercidae	*Onchocerca cervicalis*	DQ094174.1	95.08

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                              10        20        30        40        50        60        70
                         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
ON564731.1 D. invaginatus (ps)  TGAGGAGGTA GTGACGAAAA ATAACGTGAC CGTTCTCTTC GAGGCCGGTT ATCGGAATGG GTACAATTTA
EU004819.1 S. hamatus           .......... .......... ......A... .......... .......... .......... ..........
EU004818.1 S. laticeps          .......... .......... ......A... .......... .......... .......... ..........
KP861914.1 S. cf. laticeps      .......... .......... ......A... .......... .......... .......... ..........
MW358651.1 D. invaginatus       .......... .......... ......A... .......... .......... .......... ..........
                              80        90        100       110       120       130       140
                         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
ON564731.1 D. invaginatus (ps)  AACCAGTTAA CGAGGATCTA TGAGAGGGCA AGTCTGGTGC CAGCAGCCGC GGTAATTCCA GCTCTCAAAG
EU004819.1 S. hamatus           .......... .......... .......... .......... .......... .......... ..........
EU004818.1 S. laticeps          .......... .......... .......... .......... .......... .......... ..........
KP861914.1 S. cf. laticeps      .......... .......... .......... .......... .......... .......... ..........
MW358651.1 D. invaginatus       .......... .......... .......... .......... .......... .......... ..........
                              150       160       170       180       190       200       210
                         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
ON564731.1 D. invaginatus (ps)  TGTATATCGT CATTGCTGCG GTTAAAAAGC TCGTACTTGG ATCTGCGCCT CAGGAAGCCG GTCCGTCCTT
EU004819.1 S. hamatus           .......... .......... .......... ......G.... .......... .....GC.G- ..........
EU004818.1 S. laticeps          .......... .......... .......... ......G.... .......... .....CC.G- ..........
KP861914.1 S. cf. laticeps      .......... .......... .......... ......G.... .......... .....CC.G- ..........
MW358651.1 D. invaginatus       .......... .......... .......... ......G.... .......... .......... ..........
                              220       230       240       250       260       270       280
                         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
ON564731.1 D. invaginatus (ps)  TGGACGAGAA CTGGTCGCCT AGGCTAGTA- CTGTCGGTTT TCCCTGCGTT GCCTTGATCG GTCGCGTAGG
EU004819.1 S. hamatus           .......... ....A... ......-GTA. .......... .....A.. A.... .......... ..........
EU004818.1 S. laticeps          .......... ...G..... ......-GTA. .......... .......... ..T....... ..........
KP861914.1 S. cf. laticeps      .......... ...G..... ......-GTA. .......... .......A.. ..T....... ..........
MW358651.1 D. invaginatus       .......... .......... ......-... .......... .......... .......... ..........
                              290       300       310       320       330       340       350
                         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
ON564731.1 D. invaginatus (ps)  GTGGCCGGCG AGTTTACCTT GAAAAAATTA GAGTGCTCAA CGCGGGCTGA CGCCTGAATA CTCGTGCATG
EU004819.1 S. hamatus           ...A...... .......... .......... .......... .......... .......... ..........
EU004818.1 S. laticeps          .......... .......... .......... .....T.... .......... .......... ..........
KP861914.1 S. cf. laticeps      .......... .......... .......... .....T.... .......... .......... ..........
MW358651.1 D. invaginatus       .......... .......... .......... .......... .......... .......... ..........
                              360       370       380       390       400       410       420
                         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
ON564731.1 D. invaginatus (ps)  GAATAATGAA ATAGGATCTC GGTTCTATAT TGTTGGTTTT CTGATCTGAG ATAATGGTTA AGAGGGACGG
EU004819.1 S. hamatus           .......... .......... ......T. .......... .......... .......... ..........
EU004818.1 S. laticeps          .......... .......... ......T. .......... .......... .......... ..........
KP861914.1 S. cf. laticeps      .......... .......... ......T. .......... .......... .......... ..........
MW358651.1 D. invaginatus       .......... .......... ......T. .......... .......... .......... ..........
                              430       440       450       460       470       480       490
                         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
ON564731.1 D. invaginatus (ps)  ACGGGGGCAT TCGTATCGCT GCGTGAGAGG TGAAATTCTT GGACCGTAGC GAGACGCCCG ACTGCGAAAG
EU004819.1 S. hamatus           .......... .......... .......... .......... .......... .......... ..........
EU004818.1 S. laticeps          .......... .......... .......... .......... .......... .......... ..........
KP861914.1 S. cf. laticeps      .......... .......... .......... .......... .......... .......... ..........
MW358651.1 D. invaginatus       .......... .......... .......... .......... .......... .......... ..........
                              500       510       520       530       540       550       560
                         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
ON564731.1 D. invaginatus (ps)  CATTTGCCAA GAATGTCTTC ATTAATCAAG AACGAAAGAC AGAGGTTCGA AGGCGATCAG ATACCGCCCT
EU004819.1 S. hamatus           .......... .......... .......... ......T. .......... .......... ..........
EU004818.1 S. laticeps          .......... .......... .......... ......T. .......... .......... ..........
KP861914.1 S. cf. laticeps      .......... .......... .......... ......T. ...Y... .......... ..........
MW358651.1 D. invaginatus       .......... .......... .......... ......T. .......... .......... ..........
                              570       580       590       600       610       620       630
                         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
ON564731.1 D. invaginatus (ps)  AGTTCTGACC GTAAACGATA CCAACTAGCG TTCCGTCGGT GGTAAATACG CCTTGACGGG CAGCTTCCCG
EU004819.1 S. hamatus           .......... .......... .......... .......... .......... .......... ..........
EU004818.1 S. laticeps          .......... .......... .......... .......... .......... .......... ..........
KP861914.1 S. cf. laticeps      .......... .......... .......... .......... .......... .......... ..........
MW358651.1 D. invaginatus       .......... .......... .......... .......... .......... .......... ..........

ON564731.1 D. invaginatus (ps)  G
EU004819.1 S. hamatus           .
EU004818.1 S. laticeps          .
KP861914.1 S. cf. laticeps      .
MW358651.1 D. invaginatus       .
```

Fig. 5. Partial 18S rRNA gene sequence alignment of *Desportesius invaginatus* with the most closely related species (Only variable sites are given). Bases that are similar to those in the initial sequences are shown by dots, whereas gaps are indicated by dashes. *(PS mentioned specimen in the present study).*

esophagus, the length of the spicules (right spicule is longer than the left one), and the arrangement of the caudal papillae (four pairs of preanal and five pairs of postanal papillae). Wong and Anderson (1986), Varjabedian (2006), Abou Shafeey et al. (2018), and Lee et al. (2021) all described *D. invaginatus* in very few reports. Additionally, *D. invaginatus* has been observed in several Ciconiiformes hosts, including *Bubulcus ibis* (Egypt, India, and Taiwan), *Egretta garzetta* (France), *Egretta rufescens* (USA) (Wong and Anderson, 1986).

The taxa within the genus *Desportesius* have insufficient descriptions, according to Wong and Anderson (1986). The precise identification of nematodes within the Acuariidae at a specific level has

recently been made possible utilization of molecular approaches that employ the ribosomal gene regions as genetic markers (Lee et al., 2021). The nuclear ribosomal DNA 18S rRNA gene was used to study inter- and intraspecific relationships to identify *Desportesius* specimens, which agreed with Choi et al. (2014) and Kim et al.,(2018) who stressed that this genetic marker is highly repeated and contains variable regions that are flanked by more conserved regions. The 18S rRNA sequences of specimens were separated from other *Synhimantus* species, which agreed with Chabaud and Petter (1959) and Lee et al. (2021) who regarded this as the morphology of the cordon and the position of the vulva opening. Genetic diver-
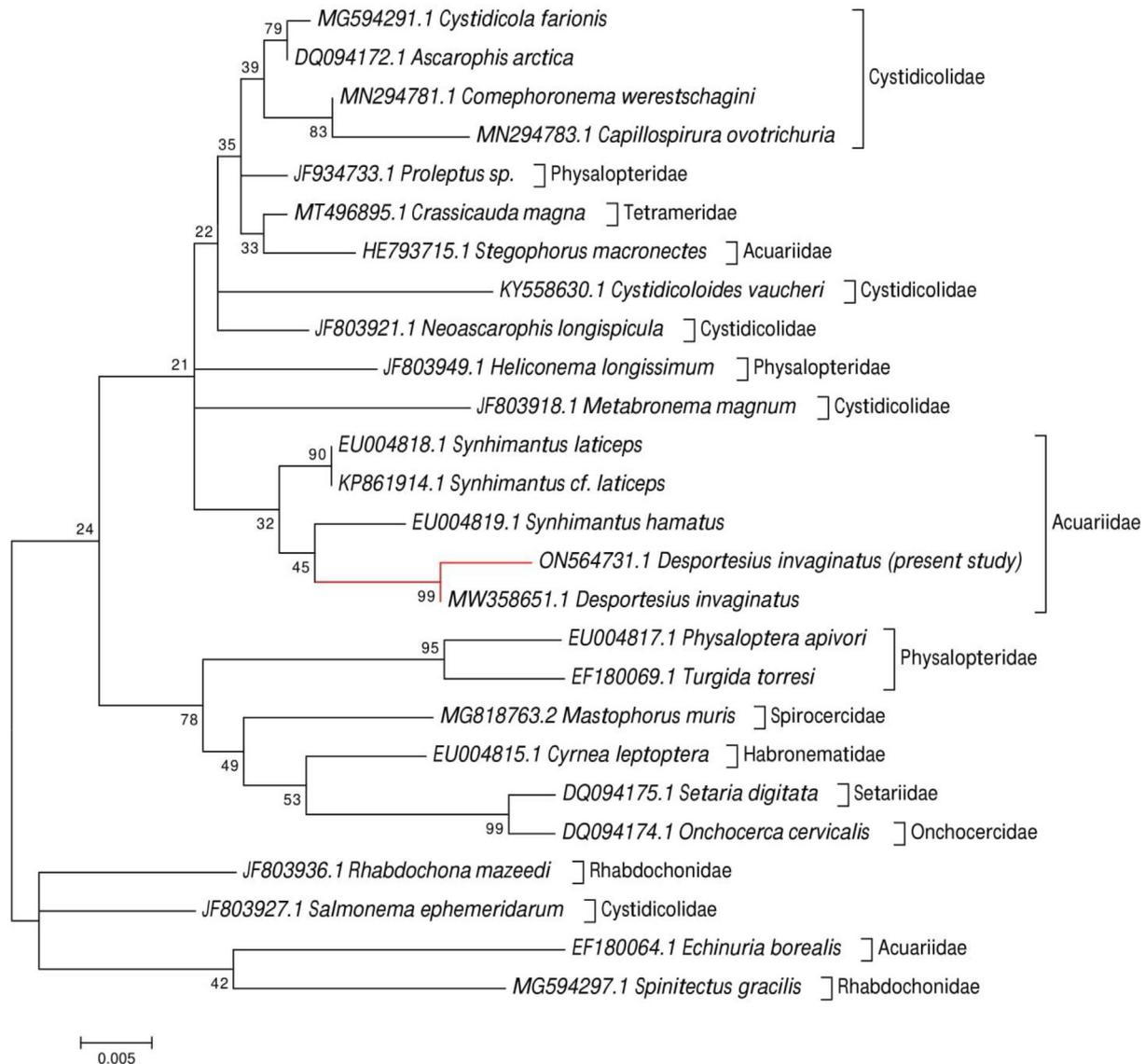
Fig. 6. Molecular phylogenetic analysis was done by the ML method for the 18S rRNA gene region based on the Tamura-Nei model. The tree with the highest log likelihood (-2122.15) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with a superior log-likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

gence of *Desportesius* specimens suggests their conspecific relationship with *S. hamatus* (EU004819.1) and *S. laticeps* (EU004818.1 and KP861914.1), respectively. Additionally, there was a strong relationship to the *D. invaginatus* specimens that had already been submitted on GenBank (MW358651.1).

7. Conclusion

Morphological and molecular phylogenetic data confirm the existence and placement of *Desportesius invaginatus* within the Acuariidae. It has been determined that this is the second genetic record for *Desportesius* species been discovered. Future research should employ more samples and genetic markers to further understand this group of acuariid parasites.

Conflict of interest

The author(s) declare that they have no conflict of interest regarding the content of this article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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