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# Morphological and molecular studies of the nematode parasite *Heterakis gallinarum* (*Heterakidae*) infecting the cattle egret *Bubulcus ibis* (*Ardeidae*)

[*Estudos morfológicos e moleculares do parasita nematoide Heterakis gallinarum (Heterakidae) que infecta a garça-vaqueira Bubulcus ibis (Ardeidae)*]

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## ABSTRACT

Parasites infecting migratory birds all over the world are still under investigation. The identification of parasitic taxa infecting ardeids was done concerning their morphological and morphometric features. A total of 20 *Bubulcus ibis* (*Ardeidae*) specimens were collected and investigated for nematode parasites. Only one nematode species, belonging to the *Heterakidae* family, has been identified, with a prevalence rate of 40% (8/20) among infected egrets. The *Heterakis* species isolated from the lumen of the ceca of the egret host is morphologically and morphometric compatible with *Heterakis gallinarum*. Additionally, utilizing the partial small subunit ribosomal RNA (18S rRNA) and mitochondrial cytochrome c oxidase subunit I (COI) genes, maximum parsimony based on the Tamura-Nei model was used to infer the phylogeny of the recovered *Heterakis* species. The query sequences revealed 99.61% and 97.11% identities for the 18S (MK844591.1) and COI (MF066715.1) genes of the previously mentioned *H. gallinarum*. In addition to clarifying several morphological features of *H. gallinarum*, this study also provided new DNA data for this species. The combination of morphological and molecular data could be helpful to other veterinaries in finding a way to treat and control this infection in the cattle egret.

Keywords: host-specificity, parasite description, phylogenetic confirmation

## RESUMO

*Os parasitas que infectam as aves migratórias em todo o mundo ainda estão sendo investigados. A identificação dos táxons parasitas que infectam os ardeídeos foi feita com base em suas características morfológicas e morfométricas. Um total de 20 espécimes de Bubulcus ibis (Ardeidae) foi coletado e investigado quanto a parasitas nematoides. Apenas uma espécie de nematoide, pertencente à família Heterakidae, foi identificada, com uma taxa de prevalência de 40% (8/20) entre as garças infectadas. A espécie Heterakis isolada do lúmen do ceco do hospedeiro garça é morfológica e morfometricamente compatível com Heterakis gallinarum. Além disso, utilizando os genes parciais da subunidade pequena do RNA ribossômico (18S rRNA) e da subunidade I da citocromo c oxidase mitocondrial (COI), a parcimônia máxima baseada no modelo Tamura-Nei foi usada para inferir a filogenia das espécies de Heterakis recuperadas. As sequências de consulta revelaram 99,61% e 97,11% de identidades para o 18S (MK844591.1) e COI (MF066715.1) do H. gallinarum mencionado anteriormente. Além de esclarecer várias características morfológicas do H. gallinarum, este estudo também forneceu novos dados de DNA para essa espécie. A combinação de dados morfológicos e moleculares pode ser útil para que outros veterinários encontrem uma maneira de tratar e controlar essa infecção na garça-vaqueira.*

*Palavras-chave: especificidade do hospedeiro, descrição do parasita, confirmação filogenética*

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## INTRODUCTION

Cattle egrets (*Bubulcus ibis*) are cosmopolitan species of birds in the family Ardeidae order Ciconiiformes (Birdlife International 2012). Parasitic diseases are one of the most frequent and important health problems that affect wild birds (Freitas *et al*., 2002). According to Borgsteede (1996), parasites affect wild animals in feeding, reproduction, or killing also. In this sense, free-living and captive wild birds can be parasitized by many helminth species, whose effects range from asymptomatic infections up to the host's death (Carneiro *et al*., 2011).

The genus *Heterakis* (Nematoda, *Heterakidae*) was established by Dujardin (1845) for the parasitic helminths that are found largely in ground-feeding birds (mainly Galliformes) and occasionally in mammals (mainly rodents) (Chabaud, 1978). About 15 species are placed in the genus, but the classification is often ambiguous due to their close resemblance, and several synonyms have arisen (Simões *et al*., 2020). The main characteristic features for the differentiation were reported for males and their specified spicule size and structure, and number and position of tail papillae. Adult worms of the genus *Heterakis* live principally in the lumen of ceca of birds (Permin and Hansen, 1998).

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 parasites (Mutafchiev *et al*., 2020).

This study aims to study the occurrence of nematodes infecting the cattle egret in Egypt and the taxonomic status of parasites was determined through morphological features and confirmed by molecular tools.

## MATERIALS AND METHODS

Twenty cattle egrets, *Bubulcus ibis* (Family *Ardeidae*), were randomly selected from the agricultural lands in the Faculty of Agricultural at Cairo University. Within 8 to 24 hr of being captured, each chosen bird was euthanized by receiving an intraperitoneal injection of a sodium pentobarbital (Blink Health, NY, US). Each specimen's alimentary canal was dissected and inspected using a stereo-dissecting microscope (Nikon SMZ18, NIS ELEMENTS software) to check for intestinal parasites. Using a tiny pipette, the collected intestinal parasites were transferred to saline solution and repeatedly washed to remove any mucus or debris that was typically adhering to their body surface, then parasites were fixed in 70% ethanol. According to Bush *et al*. (1997), parasitological term of the prevalence was estimated.

To prepare the worms for whole mounts, they were first fixed, then 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 were preserved in pure glycerin 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, then 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 photos and represented in millimeters (mm).

Using a DNeasy tissue kit© (Qiagen, Hilden, Germany), genomic DNA was extracted from ethanol-preserved samples by following the manufacturer's instructions. Through the PCR, a partial 18S rRNA and COI genes were targeted and amplified. Primers for the 18S rRNA gene were 5'-CGC GAA TRG CTC ATT ACA ACA GC-3' (forward) and 5'-GGG CGG TAT CTG ATC GCC-3' (reverse) as mentioned by Floyd *et al*. (2005), and those for COI gene were 5'-CTC CTT TGA GAA CTA GGG GGC-3' (forward) and 5'-AAC CTT AAC ACC AGT GGG CA-3' (reverse) designed in this study. On 1.5% *w/v* agarose gel in 1× Tris-acetate–EDTA (TAE) stained with SYBR green (Qiagen, Hilden, Germany), the amplicons were examined, and then observed using UV trans-illuminator.

After sequencing procedures using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Thermo Scientific Inc., USA), PCR

amplicons were submitted to automated DNA Sequencer (ABI-PRISM 310, Thermo Scientific Inc., USA). Using BioEdit 7.0.1 (Hall, 1999), a partial 18S rRNA and COI genetic sequences were aligned with those in NCBI database. Phylogenetic trees were inferred using MEGA ver.7.0 software (Kumar *et al*., 2016) based on the Maximum Likelihood (ML) analyses. The bootstrap approach with 1000 replicates was used to evaluate the ML tree.

## RESULTS

Eight out of twenty (40%) specimens of the examined cattle egret, *Bubulcus ibis*, were naturally infected with a nematode parasite with a specific site for the lumen of the ceca. This parasitic species was identified as *Heterakis gallinarum*. The intensity of infection does not exceed six in each of the parasitized egrets with a mean of 5.25.

Adult worms (Fig. 1) medium to large and creamy white. Mouth opening bordered by tri-radiate lips. Dorsal lip was somewhat broader than the sub-ventral lips. Each lip is covered with two triangular teeth of a spoon-like structure. Cephalic papillae and amphidal pores were found on the outer surface of the lips. Cuticle distinctly striated. Lateral alae extended along the whole body. The esophagus is cylindrical and slightly extended toward posterior end with a posterior bulb.

*Description of the male worms*: Body 5.20-6.97 (6.10) mm long and 0.298-0.370 (0.345) mm wide. Pharynx 0.045-0.058 (0.049) mm long. Esophagus 0.621-0.701 (0.692) mm long. Nerve ring and excretory pore located at 0.175-0.225 (0.210) mm and 0.330-0.401 (0.378) mm from anterior end of the body, respectively. The pre-cloacal sucker oval to circular with a chitinous rim, situated a short distance anterior to the cloacal opening, its diameter 0.037-0.067 (0.053) mm long and 0.031-0.042 (0.037) mm wide, located at 0.109-0.218 (0.178) mm from the cloaca and 0.634-0.711 (0.678) mm from the posterior end. Posterior extremity provided with eleven pairs of caudal papillae present and 2 unpaired: 2 pairs aside of the sucker, 1 sessile and 1 pedunculated pair of precloacal papillae, 3 pedunculated and 1 sessile pair of adcloacal papillae, and 2 pedunculated and 1 sessile pair plus 2 unpaired postcloacal papillae. Cloaca was a transverse slightly tongue-shaped slit in a distinct prominence. Two unequal spicules were protruded out at the cloacal opening, the right spicule 0.593-0.754 (0.639) mm long, and the left spicule 0.920-1.98 (1.34) mm long. Tail 0.157-0.224 (0.217) mm long.

*Description of the female worms*: Body 9.17-10.85 (10.04) mm long and 0.305-0.411 (0.379) mm wide. Pharynx 0.040-0.068 (0.053) mm long. Esophagus 0.698-0.823 (0.724) mm long. Nerve ring and excretory pore located at 0.148-0.245 (0.200) mm and 0.401-0.511 (0.470) mm from the anterior end, respectively. Vulva was situated in the middle third of the body at 1.78-5.12 (4.01) mm from the anterior extremity of the body, formed as a transverse slit with indistinct borders. The vagina was short and branched into two divergent uterine branches which were filled with embryonated eggs. Tail slender and 0.401-1.10 (0.81) mm long.

*Phylogeny of the 18S rRNA gene*: The examined nematode species' partial 18S rRNA sequence was 770 bp with a GC content of 47.4% [A(26.23% 202) | C(20.0% 154) | G(27.4% 211) | T(26.36% 203)] and deposited in GenBank under the accession number ON506414.1. The ML approach was used to align nucleotide sequence data from 27 taxa over 770 positions to produce a phylogenetic dendrogram that represented one class of Chromadorea. The overall mean distance among all sequences was 0.028. The pairwise comparison with GenBank 18S rRNA gene data sets confirmed identification of genus *Heterakis*.

The phylogenetic analysis included taxa of three superfamilies Heterakoidea (represented by two families *Heterakidae* and *Ascardiidae*), Ascaridoidea (by five families *Anisakidae*, *Toxocaridae*, *Raphidascarididae*, *Heterocheilidae*, and *Acanthocheilidae*), and Cosmocercoidea (two families *Kathlaniidae* and *Cosmocercidae*). There are different ranges of identities with comparable taxa of 99.61–94.81% for species within superfamilies Heterakoidea, 94.94–94.24% for superfamilies Ascaridoidea, and 94.11–94.29% for superfamilies Cosmocercoidea (Table 1).

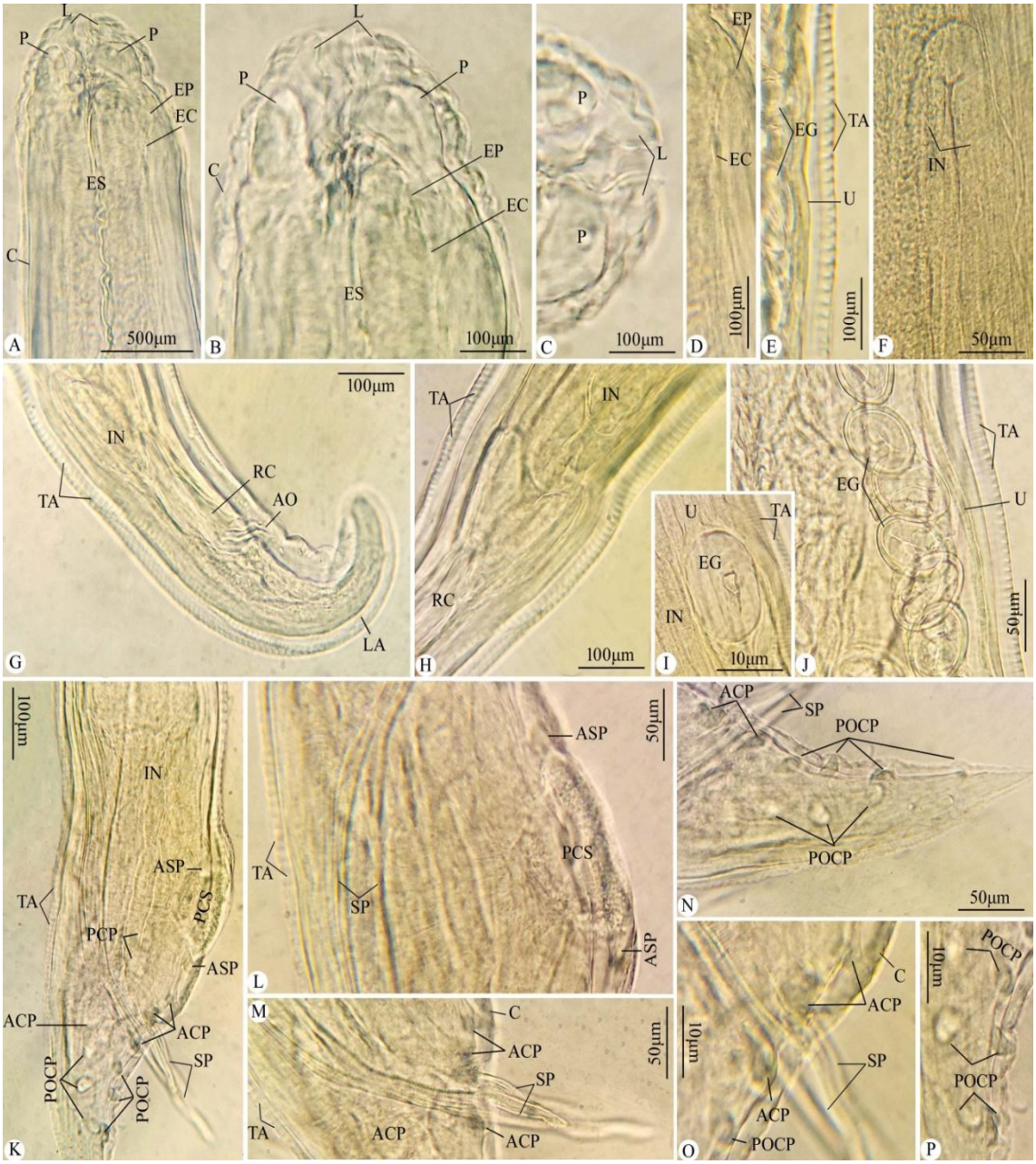


Figure 1. Light photomicrographs of *Heterakis gallinarum* infecting *Bubulcus ibis*. (A-C) Anterior region. (D) Excretory pores and related canal. (E) Cuticle with transverse annulations. (F) Intestine. (G-H) Posterior region of females. (I-J) Uterus with eggs. (K-P) Posterior region of males. Note: L, lip(s); P, papillae; EP, excretory pore; EC, excretory canal; ES, esophagus; C, cuticle; U, uterus; TA, transverse annulations; EG, eggs; IN, intestine; RC, rectum; AO, anal opening; PCS, precloacal sucker; SP, spicule(s); PCP, precloacal papillae; ACP, adcloacal papillae; POCP, postcloacal papillae; LA, lateral alae, ASP, aside sucker papillae.

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

| Superfamily | Family | Species | Accession No. | % identity |
|---|---|---|---|---|
| Heterakoidea | *Heterakidae* | *Heterakis gallinarum* | MK844591.1 | 99.61 |
| | *Heterakidae* | *Heterakis gallinarum* | DQ503462.1 | 99.48 |
| | *Heterakidae* | *Heterakis spumosa* | MH571872.1 | 97.53 |
| | *Heterakidae* | *Heterakis* sp. | AF083003.1 | 94.95 |
| | *Heterakidae* | *Strongyluris calotis* | LC133190.1 | 96.50 |
| | *Ascardiidae* | *Porrocaecum depressum* | U94379.1 | 94.81 |
| | *Ascardiidae* | *Ascaridia galli* | EF180058.1 | 98.57 |
| | *Ascardiidae* | *Baylisascaris procyonis* | U94368.1 | 94.81 |
| | *Ascardiidae* | *Parascaris equorum* | U94378.1 | 94.94 |
| | *Ascardiidae* | *Toxascaris leonine* | U94383.1 | 94.81 |
| | *Ascaridiidae* | *Krefftascaris sharpiloi* | GU245692.1 | 94.81 |
| Ascaridoidea | *Anisakidae* | *Pseudoterranova decipiens* | U94380.1 | 94.68 |
| | *Anisakidae* | *Anisakis sp.* | U94365.1 | 94.55 |
| | *Anisakidae* | *Sulcascaris sulcata* | EF180080.1 | 94.42 |
| | *Anisakidae* | *Contracaecum eudyptulae* | MW131983.1 | 94.24 |
| | *Toxocaridae* | *Toxocara canis* | U94382.1 | 94.94 |
| | *Raphidascarididae* | *Iheringascaris inquies* | U94377.1 | 94.55 |
| | *Raphidascarididae* | *Hysterothylacium reliquens* | U94376.1 | 94.68 |
| | *Raphidascarididae* | *Ascaris lumbricoides* | U94366.1 | 94.81 |
| | *Raphidascarididae* | *Raphidascaris acus* | DQ503460.1 | 94.68 |
| | *Heterocheilidae* | *Dujardinascaris waltoni* | EF180081.1 | 94.42 |
| | *Acanthocheilidae* | *Mawsonascaris zhoui* | MF072706 | 94.55 |
| | *Acanthocheilidae* | *Acanthocheilus rotundatus* | MF072699 | 94.94 |
| Cosmocercoidea | *Kathlaniidae* | *Cruzia* sp. | U94371.1 | 94.29 |
| | *Cosmocercidae* | *Nemhelix bakeri* | DQ118537.1 | 94.11 |
| | *Cosmocercidae* | *Cosmocercoides pulcher* | LC018444.1 | 94.22 |

18S rRNA, small subunit ribosomal RNA; ML, maximum likelihood

The phylogenetic dendrogram showed a well-resolved distinct clade with Heterakoidea species and the recovered nematode species, particularly those belonging to the *Heterakidae* family (Fig. 2). The recovered species have high sequence identities for genera within *Heterakidae* as 99.61–94.95% for taxa of the *Heterakis* genus and 96.50% for the *Strongyluris* genus. The sequence of current species grouped with previously deposited sequences of *Heterakis gallinarum* (MK844591.1 and DQ503462.1) infecting *Gallus gallus* that inhabiting Georgia and Australia, as expected based on sequence comparisons.

*Phylogeny of the COI gene*: The partial COI sequence was 693 bp long with 35.2% as a percentage of GC content that distributed as follows: A(22.94% 159) | C(13.42% 93) | G(21.79% 151) | T(41.85% 290). Sequence was deposited with accession number ON514033.1 in GenBank database. Analysis was based on 38 chromadorean species with 679 positions to construct a suitable dendrogram. Mean distance among comparable sequences was 0.079.

The phylogenetic analysis grouped taxa of superfamilies Heterakoidea, Ascaridoidea, and Cosmocercoidea. There are different ranges of identities between the current species and taxa of superfamilies Heterakoidea to be 99.42–83.58%, 84.48% for taxa within superfamilies Ascaridoidea, and 83.58% for superfamilies Cosmocercoidea species (Table 2).

The phylogenetic dendrogram showed a well-resolved distinct clade with species within superfamilies Heterakoidea species, particularly those taxa included within the *Heterakidae* family (Fig. 3). The recovered species have high sequence identities with lower divergence values for *Heterakis* species as 99.42 – 97.11% for *Heterakis gallinarum*, 88.29 – 87.87% for *Heterakis indica*, and 88.92 – 88.30% for *Heterakis beramporia*. The sequence of the current species grouped with the previously deposited COI sequences of *Heterakis gallinarum* (LC592851.1 and LC592866.1) infecting the ceca of *Gallus gallus* that was collected previously from Bangladesh.

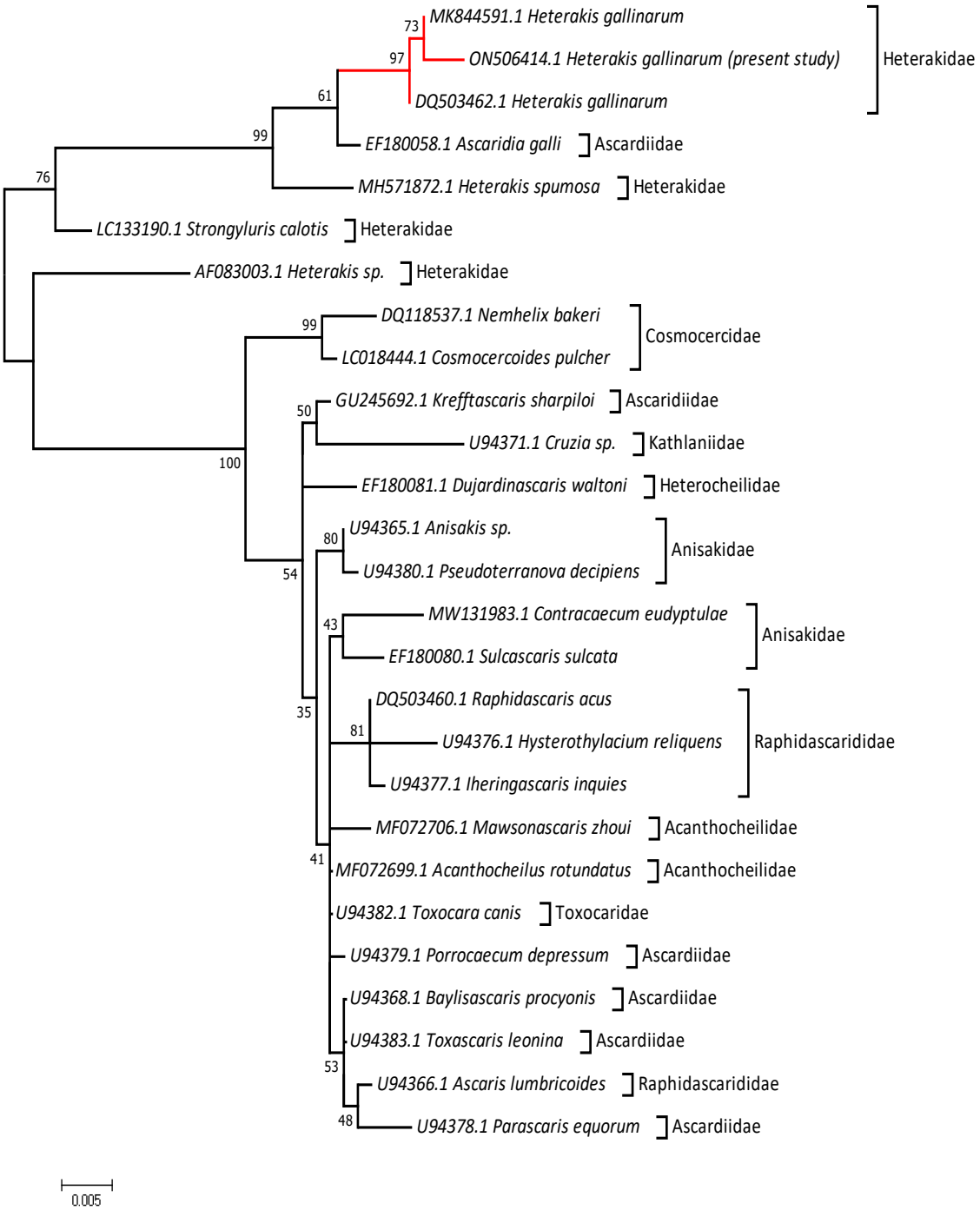


Figure 2. Molecular phylogenetic analysis was done by ML method for the 18S rRNA gene region based on the Tamura-Nei model. The tree with the highest log likelihood (-2015.65) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms 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.

Table 2. GenBank accession numbers for COI sequences used in ML analysis

| Superfamily | Family | Species | Accession No. | % identity |
|---|---|---|---|---|
| | *Heterakidae* | *Heterakis gallinarum* | MF066715.1 | 97.11 |
| | *Heterakidae* | *Heterakis gallinarum* | LC592853.1 | 97.84 |
| | *Heterakidae* | *Heterakis gallinarum* | LC592857.1 | 97.98 |
| | *Heterakidae* | Heterakis gallinarum | KP308361.1 | 98.12 |
| | *Heterakidae* | *Heterakis gallinarum* | KP308336.1 | 98.12 |
| | *Heterakidae* | *Heterakis gallinarum* | KP308344.1 | 98.12 |
| | *Heterakidae* | *Heterakis gallinarum* | KP308363.1 | 98.12 |
| | *Heterakidae* | *Heterakis gallinarum* | KP308315.1 | 98.27 |
| | *Heterakidae* | *Heterakis gallinarum* | KP308322.1 | 98.27 |
| | *Heterakidae* | *Heterakis gallinarum* | KP308327.1 | 98.27 |
| | *Heterakidae* | *Heterakis gallinarum* | KP308337.1 | 98.27 |
| | *Heterakidae* | *Heterakis gallinarum* | KP308350.1 | 98.27 |
| | *Heterakidae* | *Heterakis gallinarum* | KP308352.1 | 98.27 |
| | *Heterakidae* | *Heterakis gallinarum* | KP308354.1 | 98.27 |
| | *Heterakidae* | *Heterakis gallinarum* | KP308355.1 | 98.27 |
| | *Heterakidae* | *Heterakis gallinarum* | KP308357.1 | 98.27 |
| | *Heterakidae* | *Heterakis gallinarum* | KP308362.1 | 98.27 |
| Heterakoidea | *Heterakidae* | *Heterakis gallinarum* | KP308325.1 | 98.27 |
| | *Heterakidae* | *Heterakis gallinarum* | KP308329.1 | 98.27 |
| | *Heterakidae* | *Heterakis gallinarum* | KP308349.1 | 98.27 |
| | *Heterakidae* | *Heterakis gallinarum* | LC592851.1 | 99.13 |
| | *Heterakidae* | *Heterakis gallinarum* | LC592866.1 | 99.42 |
| | *Heterakidae* | *Heterakis indica* | LC592873.1 | 87.87 |
| | *Heterakidae* | *Heterakis indica* | LC592874.1 | 88.08 |
| | *Heterakidae* | *Heterakis indica* | LC592871.1 | 88.19 |
| | *Heterakidae* | *Heterakis indica* | LC592875.1 | 88.19 |
| | *Heterakidae* | *Heterakis indica* | LC592869.1 | 88.29 |
| | *Heterakidae* | *Heterakis indica* | LC592872.1 | 88.29 |
| | *Heterakidae* | *Heterakis beramporia* | LC592902.1 | 88.30 |
| | *Heterakidae* | *Heterakis beramporia* | LC592868.1 | 88.40 |
| | *Heterakidae* | *Heterakis beramporia* | LC592901.1 | 88.40 |
| | *Heterakidae* | *Heterakis beramporia* | LC592900.1 | 88.50 |
| | *Heterakidae* | *Heterakis beramporia* | KU529972.1 | 88.61 |
| | *Heterakidae* | *Heterakis beramporia* | LC592867.1 | 88.92 |
| | *Ascaridiidae* | *Ascaridia galli* | KT613901.1 | 86.29 |
| Ascaridoidea | *Anisakidae* | *Anisakis brevispiculata* | KJ786254.1 | 84.48 |
| Cosmocercoidea | *Cosmocercidae* | *Cosmocercoides sp.* | LC201938.1 | 83.58 |

COI, cytochrome c oxidase I; ML, maximum likelihood.

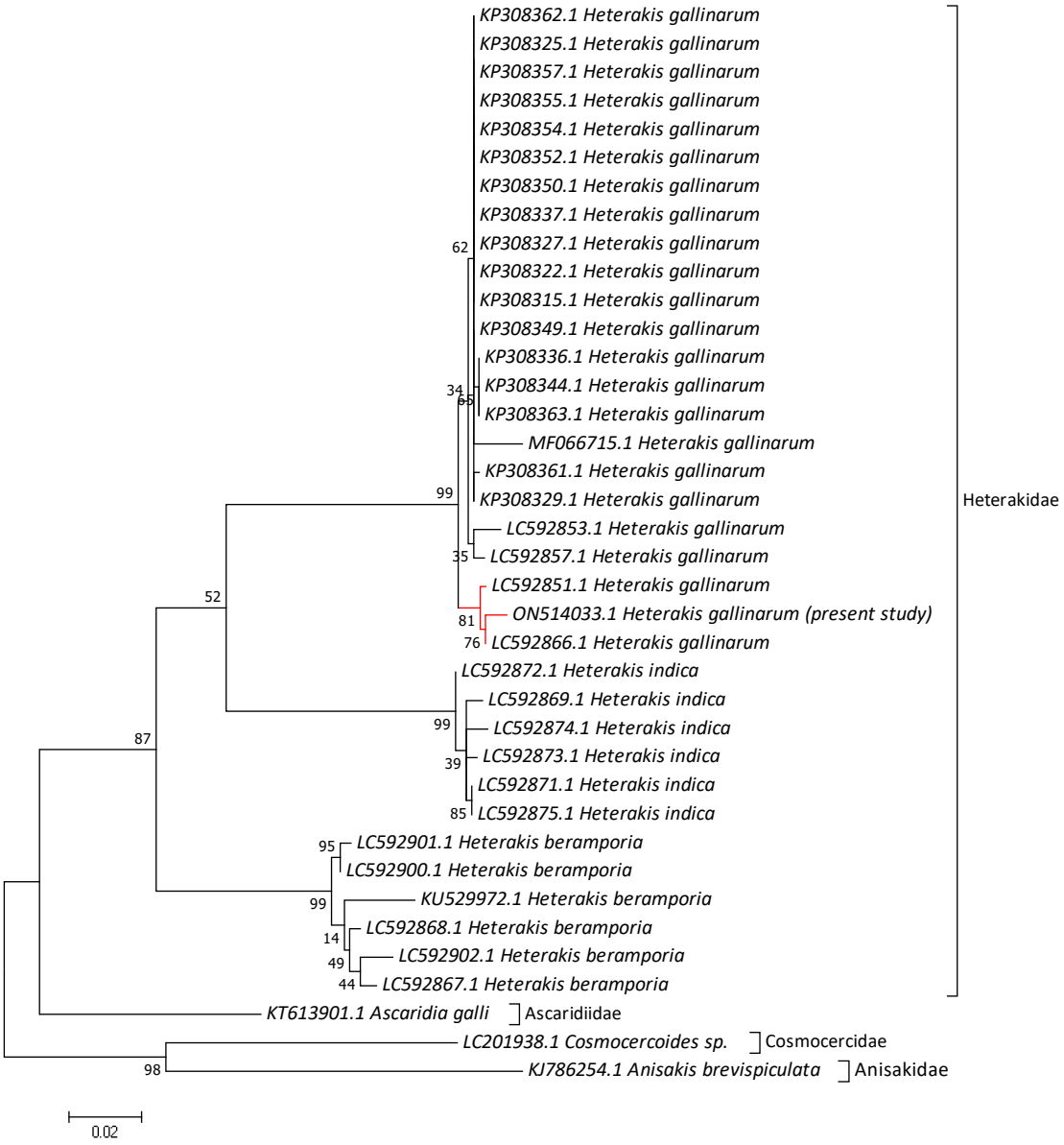


Figure 3. Molecular phylogenetic analysis was done by the ML method for the COI gene region based on the Tamura-Nei model. The tree with the highest log likelihood (-2867.02) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms 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.

## DISCUSSION

Nematodes of the genus *Heterakis* Dujardin, 1845 are common and generalist parasites within the digestive tract of various bird species (Cupo and Beckstead, 2019). In this study, *Heterakis gallinarum* was recovered from the ceca of 40% of the examined *Bubulcus ibis*. Similar findings were documented in Molla *et al*. (2012) who recorded a rate of infection to be 39.62% for this *Heterakis* species in birds from North Gondar off Ethiopia. A possible explanation for this rate of infection could be explained by the presence of favorable conditions (such as temperature and

hygrometric degree) for the development of *Heterakis gallinarum* eggs.

Identification of the recovered *Heterakis* parasite was performed by morphological comparison and molecular analysis via DNA sequencing that could assist in the process of identifying the species and building a *Heterakis* species, which agreed with the previous studies of Wang *et al*. (2016), Cupo and Beckstead (2019), and Simões *et al*. (2020). In the current study, the specimens analyzed, present the general characteristic features described for the genus *Heterakis*, especially those for male worms, such as spicule structures and lengths, the number and position of the caudal papillae, the presence of the precloacal sucker and its relative length to the cloacal aperture, and the length of the tail. These criteria are considered suitable taxonomic characters to differentiate species within the genus *Heterakis*, as reported by (Simões *et al*., 2020). Furthermore, it was found that the females had eggs at different stages of the development. Findings of this study corroborate Saunders *et al*. (2000) for *Heterakis gallinarum* which eliminates non-embryonic eggs in the external environment, where larvae development occurs. Except for minor differences in the measurements, morphological features of the detected nematode species in this study were in close agreement with the earlier descriptions of *Heterakis gallinarum* by Schrank (1788) and the following studies by Park and Shin (2010), Tanveer *et al*. (2015), Wheeb *et al*. (2015), Yevstafieva *et al*. (2018), Simões *et al*. (2020). The possible hypothesis for the difference in measurements of the worms might be attributed to the birds, from which the parasites were collected, and the methods of preparation of the parasites for examination, which is consistent with the opinion of Al-Moussawi (2016).

The morphology and ecology of the parasite have traditionally provided basis for taxonomy of nematode parasites (Bobrek *et al*., 2019). Recent advancement in molecular techniques has opened opportunities to develop novel parasitological diagnostic tools, which are more sensitive and specific compared with conventional diagnostic approaches (Tarbiat *et al*., 2021). In the present study, two genes of 18S rRNA and COI were targeted to be amplified and sequenced for the recovered parasite species. Until now, there are few records for sequences of the ribosomal genes for the previous *Heterakis* species deposited on the GenBank database, this may be regarding the hypothesis of Ley *et al*. (2005) that the failure of ribosomal genes to delineate closely allied species in certain nematode groups which limit their utility in the analysis of nematode diversity. However, there is many records for the mtCOI gene for the previous *Heterakis* species deposited on the GenBank database, this may be related to the previous studies of Floyd *et al*. (2002), and Elsasser *et al*. (2009) that the mitochondrial genes were considered as the potential markers for species discrimination. Also, Derycke *et al*. (2010) stated that the barcode region of COI has delivered the species-level resolution in certain nematode lineages.

The phylogenetic analyses showed the present specimen formed a branch with the *Heterakis* parasites, indicating a closer relationship within the genus *Heterakis* with strong support, but a distinct distance from species that had been reported, this result is consistent with Bobrek *et al*. (2019). Also, this relationship may be related to the high GC content which would increase translational efficiency in the *Heterakis* species given the positive correlation that exists between the recombination rate and GC content; this translational correspondence has been observed in *Heterakis spumosa* by Šnábel *et al*. (2014). In ML analyses, present findings showed strong support that both *Ascaridia galli* and *Heterakis* species were sister taxa, similar to the previous report of Liu *et al*. (2016). The results also proposed the hypothesis that *Heterakidae* had a closer relationship with *Ascaridiidae*, and the latter family was paraphyly within Heterakoidea; the result is consistent with that based on the COI sequence previously reported by Šnábel *et al*. (2014) and Gao *et al*. (2019). According to the structure of the phylogenetic trees, results supported previous reports by Li *et al*. (2018) that superfamily Heterakoidea and Ascaridoidea were monophyly. *Heterakis* species herein is described as *Heterakis gallinarum* based on the morphology and confirmed molecularly based on the 18S rRNA and mt COI genes as a separate species with a relationship to the previously described species of *Heterakis gallinarum* infecting *Gallus gallus* with accession numbers MK844591.1 and DQ503462.1 for 18S rRNA gene and LC592851.1 and LC592866.1 for COI gene.

## CONCLUSION

The cattle egret, *Bubulcus ibis*, a novel host bird for *Heterakis gallinarum* in Egypt, was discovered in this study. The partial COI gene sequences were demonstrated to be an effective genetic marker more than the 18S rRNA gene for the heterakoid DNA barcoding. More research studies are needed to have a better understanding of parasitic nematode diversity so that future changes in the distribution of the terrestrial biota can be predicted. Combination of morphological and molecular data could be helpful to other veterinaries in finding a way to treat and control this infection in the cattle egret.

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