Arq. Bras. Med. Vet. Zootec., v.76, n.4, e13232, 2024

http://dx.doi.org/10.1590/1678-4162-13232
Original Article - Veterinary Medicine

Identification tools of *Diplostomum spathaceum* Rudolphi, 1819 (Diplostomida: Diplostomidae), a trematode parasite of herring gull (*Larus argentatus*)

[*Ferramentas de identificação do Diplostomum spathaceum Rudolphi, 1819 (Diplostomida: Diplostomidae), um trematódeo parasita da gaivota de arenque (Larus argentatus)*]

E. *Alshehri*, R. *Abdel-Gaber*[*], S. *Al-Quraishy*

Zoology Department, College of Science, King Saud University, Riyadh, Saudi Arabia

ABSTRACT

Specimens of the genus *Diplostomum* von Nordmann, 1832 (Trematoda, Diplostomidae) were collected from the intestine of the herring gull, *Larus argentatus* (Laridae), from El-Manzala Lake (Port Said City, Egypt). This parasite species was morphometrically, morphologically, and molecularly studied using the internal transcribed spacer (ITS1-5.8S-ITS2) gene region. The presence of a trematode species of *Diplostomum spathaceum* Rudolphi, 1819 (Diplostomidae) was observed in 70 % of the examined gull species. This species has generic features of the genus *Diplostomum*. Distinct criteria that discriminated this species from congeners were the division for body parts, the egg-shaped forebody with a smaller length than the hind body, the ventral sucker being smaller in size than the oral one, the position of the ventral sucker being near to the holdfast, vitellaria was compact and rarely extend anteriorly to the holdfast organ, and smaller egg size. Partial ITS1-5.8S-ITS2 sequences from diplosomite recovered in this study showed that they grouped with members of the genus *Diplostomum* and formed a monophyletic group supporting the morphological description. Findings obtained from molecular analysis are consistent with data from morphological classification where the parasite recorded was morphologically similar to *Diplostomum spathaceum* with a first record in Egyptian gulls.

Keywords: gulls, diplostomidae, morphology, phylogeny

RESUMO

Espécimes do gênero Diplostomum *von Nordmann, 1832 (Trematoda, Diplostomidae) foram coletados do intestino da gaivota de arenque, Larus argentatus (Laridae), coletada no lago El-Manzala (cidade de Port Said, Egito). Essa espécie de parasita foi estudada morfometricamente e morfologicamente, bem como molecularmente, usando a região do gene espaçador transcrito interno (ITS1-5.8S-ITS2). A presença de uma espécie de trematoda de Diplostomum spathaceum Rudolphi, 1819 (Diplostomidae) foi observada em 70% das espécies de gaivotas examinadas. Essa espécie tem características genéricas do gênero* Diplostomum. *Os critérios distintos que discriminaram essa espécie das congêneres foram a divisão das partes do corpo, o corpo dianteiro em forma de ovo com comprimento menor do que o corpo traseiro, a ventosa ventral de tamanho menor do que a oral, a posição da ventosa ventral próxima ao suporte, a viterlária compacta e raramente estendida anteriormente ao órgão do suporte e o tamanho menor do ovo. As sequências parciais ITS1-5.8S-ITS2 de diplostomídeos recuperadas neste estudo mostraram que eles se agruparam com membros do gênero Diplostomum e formaram um grupo monofilético que apoia a descrição morfológica. Os resultados obtidos com a análise molecular são consistentes com os dados da classificação morfológica, em que o parasita registrado era morfologicamente semelhante ao* Diplostomum spathaceum, *com um primeiro registro em gaivotas egípcias.*

Palavras-chave: gaivotas, diplostomídeos, morfologia, filogenia

INTRODUCTION

Gulls, particularly *Larus* species, are abundant and adaptable birds of the family Laridae with a worldwide cosmopolitan distribution (Burger and Gochfeld, 1996). These birds are susceptible to infection with a wide variety of intestinal flukes (Lee *et al*., 2020). Despite the importance of these aquatic birds as a source of protein, they also transport parasitic infections to fish and domestic birds, and even to humans (Yaseen and Abdullah, 2018). Diplostomidae Poirier, 1886 is a family of trematodes in the order Diplostomida,

*Corresponding autor: rabdelgaber@ksu.edu.sa
Submitted: January 21, 2024. Accepted: February 20, 2014.

with 42 genera split among four subfamilies (Heneberg *et al*., 2020). Diplostomes have a complex life cycle, which includes development of larval stages in 2 intermediate hosts (cercaria in lymnaeid snails and metacercaria in a wide range of freshwater fish) and adult flukes in the digestive tract of various fish-eating bird species, such as gulls (Karvonen, 2012).

The type-genus *Diplostomum* von Nordmann, 1832 (subfamily Diplostominae) is a diverse group of digenean parasites with a complex taxonomy (Niewiadomska, 2010; Georgieva *et al*., 2013; Blasco-Costa *et al*., 2014; Faltýnková *et al*., 2016; Hoogendoorn *et al*., 2020) due to it includes numerous cryptic species-level lineages (Georgieva *et al*., 2013; Faltýnková *et al*., 2014; Selbach *et al*., 2015; Soldánová *et al*., 2017; Gordy and Hanington, 2019; Hoogendoorn *et al*., 2020). Previous studies focused on various topics related to ecology, host-parasite relationships, systematics, and taxonomy of *Diplostomum* species (Galazzo *et al*., 2002; Karvonen *et al*., 2006; Seppälä *et al*., 2008; Niewiadomska, 2010; Locke *et al*., 2015; Kudlai *et al*., 2017; Vivas Muñoz *et al*., 2021). Identification of *Diplostomum* species has been carried out based on the morphological data of the adult specimens since the larval stages often lack reliable distinguishing morphological characters (Höglund and Thulin, 1992; Galazzo *et al*., 2002; Pérez-del-Olmo *et al*., 2014; Sitko and Rząd, 2014; Yaseen and Abdullah, 2018; Lee *et al*., 2020; Faltýnková *et al*., 2022).

Molecular characterization of larval and adult diplostomes utilizing data from rRNA markers has proven to be useful in supplementing their morphology-based identification (Galazzo *et al*., 2002; Cavaleiro *et al*., 2012; Ndeda *et al*., 2013; Blasco-Costa *et al*., 2014; Faltýnková *et al*., 2014; Pérez-del-Olmo *et al*., 2014; Brabec *et al*., 2015; Aksenova *et al*., 2016; Achatz *et al*., 2022; Khoshnaw and Abdullah, 2023). However, most genetic sequences originate from the larval stages of diplostomes not accurately identified morphologically to species-level which prevents the resolution of the systematics of *Diplostomum* (Hoogendoorn *et al*., 2020). The barcode region of the mitochondrial (mt) cytochrome c oxidase I (COI) gene has also been largely utilized as a tool elucidating life cycles and recognition of cryptic species diversity within *Diplostomum* (Blasco-Costa *et al*., 2014; Brabec *et al*., 2015; Kudlai *et al*., 2017; Locke *et al*., 2020; Achatz *et al*., 2022; Barata *et al*., 2022, 2023; Faltýnková *et al*., 2022; Sokolov *et al*., 2023).

Although many reports have been published on helminths of various gull species from many parts of the world, few studies are available in Egypt (Abdel-Aal *et al*., 2001; Abdel-Aal and El-Sayed, 2003; Ghattas, 2004; Tadros *et al*., 2013). The present study was carried out to shed more light on the *Diplostomum* species infecting herring gull (*Larus argentatus*) using integrative taxonomy with morphological description of the adult species associated with molecular analysis.

MATERIAL AND METHODS

A total of 20 birds of herring gull, *Larus argentatus* (Laridae), were hunted from February through September 2019 from the shore of El-Manzala Lake (Gamil Outlet in Port Said City, Egypt), and then moved to the laboratory for examination. Each bird was euthanized, within 8-24hr of capture, by receiving an intraperitoneal injection of sodium pentobarbital (Blink Health, NY, US). Birds were dissected, and the digestive tract was isolated and divided into main parts (Esophagus, proventriculus + gizzard, intestine, intestinal caeca, rectum, and cloaca) then each part was placed in 0.9% saline solution and examined under a dissecting microscope (Nikon SMZ18, NIS ELEMENTS software). The worms were collected and counted. The prevalence and intensity of infection were analyzed statistically following Bush *et al*. (1997) guidelines. Worms were fixed with 10% formalin, stained with Semichon's aceto-carmine, dehydrated in the ethyl alcohol series, cleared with xylene, and mounted in Canada balsam. Photomicrographs were taken with a Leica DM 2500 microscope (NIS ELEMENTS software, ver. 3.8). All measurements in the description and table were made with an Olympus ocular micrometer then given in micrometers and expressed as range followed by mean in parentheses.

gDNA was extracted from the ethanol-preserved worms by using procedure of the manufacturer's Qiagen DNeasy^{TM} tissue kit. PCR reaction was performed to amplify the gDNA target using specific primers for the ITS1-5.8S-ITS2 gene: the forward primer D1 (5`-AGG AAT TCC TGG TAA GTG CAA G-3`) and the reverse one D2 (5`-CGT TAC TGA GGG AAT CCT GGT-3`),

designed by Galazzo *et al*. (2002). PCR reactions of 25µL were included in 2 mM $MgCl_2$, 1.25 U rTaq polymerase buffer, 2.5µL 10× rTaq DNA buffer, 0.2 mM each of dNTPs, 2.5µM of each primer, 1 µl of DNA sample and completed to 25µL with dist. H_2O. The thermal cycle was performed in a thermocycler (BioRad) with the following conditions: 94°C for 2 min, then 30 cycles of 1 min at 94°C, 1 min at 56°C, and 2 min at 72°C and finally post-PCR extension for 5 min at 72°C. PCR products were examined using 1.5% w/v agarose gel (Sigma-Aldrich, Missouri, USA) in 1× Tris-acetate-EDTA (TAE) and stained with SYBR Safe DNA gel dye (Thermo Fischer Scientific, Ottawa, Canada) against the GeneRuler 100bp Plus ready-to-use DNA ladder (Fermentas, Lithuania). BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) used for sequencing PCR amplicons with 310 Automated DNA Sequencer (Applied Biosystems, USA). ITS1-5.8S-ITS2 sequences were deposited in GenBank™ and compared with those data available in the NCBI database. Phylogenetic analysis was performed in MEGA X (Kumar *et al*., 2018). Phylogenetic tree was constructed using maximum parsimony with 1000 replicates of bootstrapping.

RESULTS

Trematode parasites were naturally infected fourteen out of twenty (70%) specimens of the examined herring gull species, *Larus argentatus*. Adult worms were observed in the intestinal region of infected gull and identified as *Diplostomum spathaceum* Rudolphi, 1819 and described in Figure (1) with measurements for different body parts in Table 1.

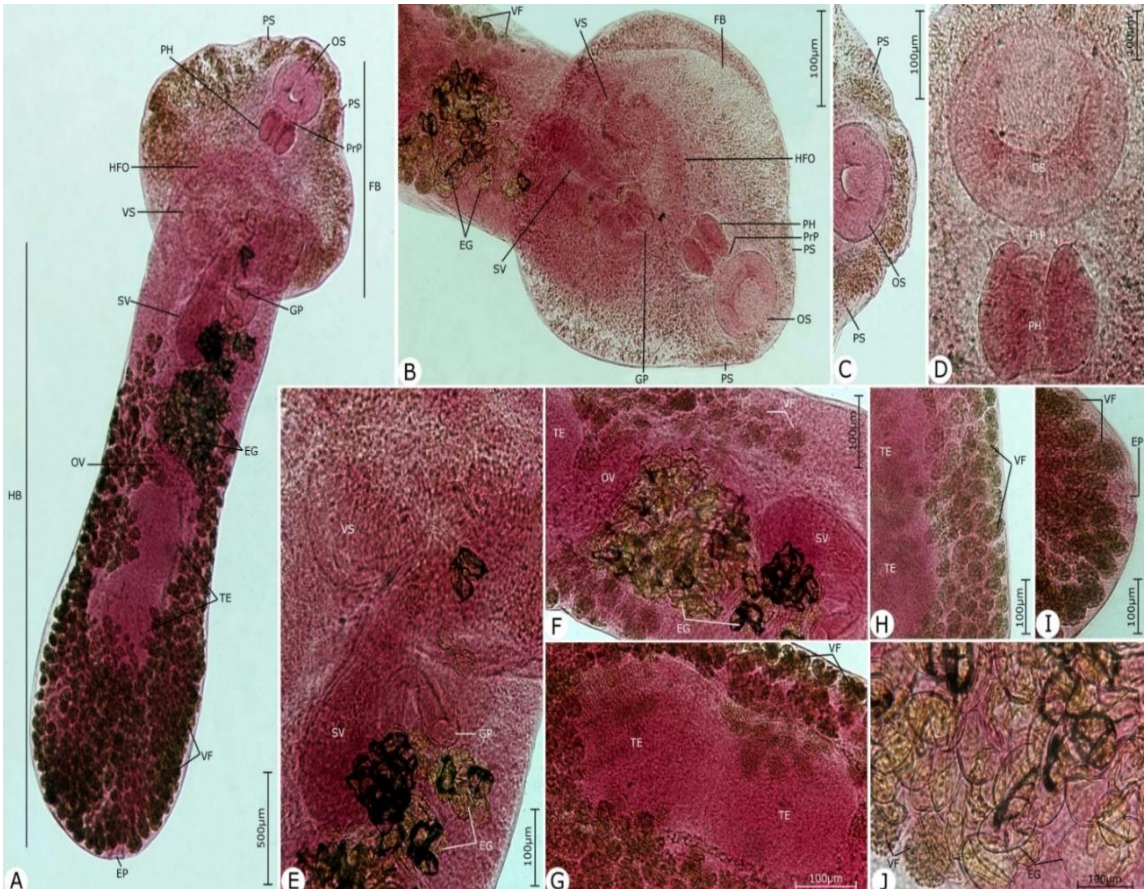

Figure 1. Photomicrographs of the adult *Diplostomum spathaceum* infecting *Larus argentatus*. (A) Whole mount preparation. (B-J) High magnifications for different body parts showing: (B-E) Forebody region. (F-J) Hindbody region. Note: EG, Eggs; EP, Excretory pore; FB, Forebody; GP, Genital pore; HB, Hindbody; HFO, Holdfast organ; OS, Oral sucker; OV, Ovary; PH, Pharynx; PrP, Pre-Pharynx; PS, Pseudosucker; SV, Seminal vesicle; TE, Testes; VS, Ventral sucker; VF, Vitelline follicles.

The body was bipartite with a total length of 1890-2021 (1984). The forebody was oval, dorso-ventrally flattened, 725-1062 (832) long with 40-45 (42)% of the total body length, with maximum width 483-682 (509) at the level of the holdfast organ. The hind body was elongated-oval, narrower anteriorly, measured 1131-1297 (1204) long, with a maximum width of 325-496 (437) at the level of the anterior testis. The oral sucker was ventro-subterminal, subspherical, measured 68-98 (82) long and 65-96 (80) wide.

The ventral sucker was sub-globular, situated just anteriorly to the mid-forebody, 60-91 (73) long and 75-96 (81) wide. Pseudosuckers were two in number, present on each side of the oral sucker, measured 102-143 (131) long and 39-58 (51) wide. Holdfast organ was large, sub-globular, close to the ventral sucker and measured 154-243 (236) long and 223-297 (257) wide. Prepharynx was short. The pharynx was oval, measured 51-84 (63) long and 40-54 (47) wide. Esophagus was indistinct; the caeca was narrow and directed posteriorly to the level of the holdfast organ.

The testes were two in number, located in the posterior half of the hind body; the anterior testis measured 167-198 (186) long and 150-218 (176) wide; posterior testis was 187-298 (283) long and 235-401 (354) wide. Seminal vesicle was voluminous.

Table 1. Comparative measurements for the present *Diplostomum spathaceum* with those described previously

Comparable parameters	Krause (1914)	Dubois (1938)	Cichoelas (1961)	Dubois (1970)			Sweeting (1976)	Dick and Rosen (1981)
Host species	*L. argentatus*, *L. fuscus*, *L. canus*	*L. argentatus*	*L. ridibundus*	*Larus* spp.	*Larus* spp.	*Larus* spp., *Sterna* spp., *Rissa tridactyla*, *Alca torda*	*L. argentatus*	*L. argentatus*
Locality	BRD	-	Baltic	USA	USA	-	-	-
Body	1500-3370	1250-4450	-	3250	3400	1250-4450	737-2277	2730-3484
Forebody	600-1360 × 360-690	640-1800 × 270-960	731-1003 × 510-935	520-1620 × 450-920	820-1500 × 270-850	600-1800 × 270-960	737-862	1326-1651 × 585-884
Hind body	770-2100 × 230-710	520-3220 × 210-750	850-1105 × 306-442	520-1800 × 400-710	880-2280 × 250-810	520-3220 × 210-750	550-1416	1482-1872 × 355-637
Oral sucker	54-94 × 54-99	40-100 × 48-104	68-75	65-112 × 51-105	55-110 × 49-97	40-100 × 46-104	70-91	65-105 × 65-110
Pseudo sucker (right)	-	-	-	80-135	-	75-180	-	90-105
Pseudo sucker (left)								
Ventral sucker	54-110 × 59-140	48-101 × 48-130	-	60-120 × 73-138	60-110 × 60-135	48-110 × 48-140	98-102	75-115 × 100-125
Holdfast organ	220-410 × 240-390	125-450 × 90-432	119-165	180-360 × 150-340	110-310 × 120-320	125-450 × 90-390	200-234	225-415
Pharynx	41-81 × 46-72	48-91 × 26-75	-	51-98 × 36-64	50-94 × 30-79	39-91 × 25-75	-	65-95 × 50-70
Testis (anterior)	170-340 × 280-520	95-460 × 130-560	-	200-435 × 330-630	170-420 × 220-490	95-460 × 130-560	494-273	325-400 × 285-500
Testis (posterior)	210-400 × 320-590	90-485 × 190-650	-	225-500 × 370-610	220-560 × 240-560	90-485 × 190-650	385-519	320-425 × 355-510
Ovary	77-130 × 100-170	50-205 × 70-235	-	69-170 × 123-225	80-190 × 90-200	50-205 × 70-235	136-197	90-170 × 105-195
Egg	-	-	-	-	-	84-115 × 52-76	-	-

Table 1. Continued …

Comparable parameters	Niewiadomska (1984)	Sonin (1986)	Brady (1989)	Pérez-del-Olmo *et al*. (2014)	Yaseen and Abdullah (2018)	Lee *et al*. (2020)	Present study
Host species	*L. fuscus*, *L. ridibundus*	*Larus* spp.	*L. argentus*	*L. argentatus*, *L. ridibundus*	*L. cachinnans*	*L. argentatus*	*L. argentatus*
Locality	Poland	Eurasia	Scotland	Spain	Iraq	Korea	Egypt
Body	Up to 4000	3830	1313-2550 (1952)	1971-2189	2440-3800 (3100)	1750-2000	1890-2021 (1984)
Forebody	1110-1480 × 590-850	850-1630 × 400-1090	440-1000 (718) × 430-610 (529)	782-1155 × 504-726	900-1350 (106) × 600-800 (700)	680-850 × 650-700	725-1062 (832) × 483-682 (509)
Hind body	1560-2920 × 560-660	1090-2800 × 325-770	775-1550 (1222) × 300-470 (371)	1252-1368 × 387-575	1420-2500 (2090) × 580-650 (600)	1080-1100 × 500-520	1131-1297 (1204) × 325-496 (437)
Oral sucker	57-95 × 74-102	55-100 × 60-111	60-88 (75) × 63-90 (76)	71-93 × 70-92	60-110 (80) × 80-160 (950)	60-65 × 75-100	68-98 (82) × 65-96 (80)
Pseudo sucker (right)	102-153	70-130	-	109-155 × 44-62	90-120 (110) × 50-80 (60)	-	102-143 (131) × 39-58 (51)
Pseudo sucker (left)					110-140 (120) × 40-70 (50)		
Ventral sucker	78-95 × 89-102	65-110 × 65-130	60-85 (74) × 73-100 (90)	65-95 × 80-99	80-150 (89) × 90-120 (110)	75-85 × 80-100	60-91 (73) × 75-96 (81)
Holdfast organ	238-374 × 259-399	150-460 × 150-440	-	150-236 × 202-288	200-300 (270) × 210-390 (250)	175-275 × 125-160	154-243 (236) × 223-297 (257)
Pharynx	59 - 74 × 51 - 74	50-85 × 45-70	53-73 (64) × 40-53 (46)	55-89 × 45-59	60-120 (80) × 50-90 (70)	50-52 × 55-65	51-84 (63) × 40-54 (47)
Testis (anterior)	185-540 × 421-629	190-430 × 220-500	-	171-203 × 154-224	170-420 (250) × 300-550 (470)	325-350 × 140-150	167-198 (186) × 150-218 (176)
Testis (posterior)	348-592 × 466-658	220-550 × 280-550	-	190-317 × 240-399	160-530 (390) × 350-620 (520)	300-325 × 175-225	187-298 (283) × 235-401 (354)
Ovary	138-222 × 163-236	90-180 × 70-270	90-130 (114) × 90-150 (122)	87 × 83	130-200 (150) × 160-200 (180)	100-135 × 75-100	79-91 (89) × 80-99 (90)
Egg	-	-	80-100 (90)	89-99 × 61-66	110-150 (130) × 80-100 (90)	108-125 × 65-85	84-91 (87) × 56-68 (61)

Genital pore was dorso-subterminal. Ovary was sub-globular, pre-testicular, measured 79-91 (89) long and 80-99 (90) wide. Vitellarium follicular was in shape and numerous, arranged in lateral bands surrounding the holdfast organ and posterior margin of the forebody, and well-distributed on both sides of the hind body reaching to the posterior extremity of the body. Eggs were few, measured 84-91 (87) long and 56-68 (61) wide.

DNA amplification of the partial ITS1-5.8S-ITS2 gene region from *D. spathaceum* resulted in a fragment of ~847 bp. One sequence was obtained from the organism detected in the present study, which was morphologically identified as *D. spathaceum*, and deposited in GenBank under the accession number PP177554. In GenBank, there are 13 sequences belonging to the members of the Genus *Diplostomum*, which belong to the adult stages of 3 species infecting *Larus* species. *D. spathaceum* was represented by 5 sequences, *D. pseudospathaceum* was represented by 4 sequences whereas *Diplostomum* sp. was represented by 4 sequences. Sequence identity ranged between 98.47-98.58% in *D. spathaceum*,

to 97.76% in *D. pseudospathaceum*, and 97.40-97.99% in *Diplostomum* sp., respectively. Phylogenetic analysis revealed that sequences obtained in the present study clustered with the clade which included *Diplostomum* members including *D. spathaceum, D. pseudospathaceum,* and *Diplostomum* sp. with strong bootstrap support (Figure 2). Members of *Diplostomum* were distinct from those in the diplostomidid genera of *Austrodiplostomum*, *Tylodelphys*, and *Neodiplostomum* (Figure 2). *Gigantobilharzia huronensis* (EF071986.1) was used in analysis as an outgroup.

DISCUSSION

The *Diplostomum* species (family Diplostomidae Poirier, 1886) are trematode parasites that spend part of their life cycle in the intestine of fish-eating avian hosts, with *D. spathaceum* being found in gulls and terns (Höglund and Thulin, 1992; Marcogliese *et al*., 2001; Blasco-Costa and Locke, 2017).

In this study, the recovered parasite species was consistent with the diagnosis of the genus *Diplostomum* by Niewiadomska and Laskowski (2002) in distinctly bipartite body, a trilobate anterior extremity with pseudosuckers, vitelline follicles that distributed in the prosoma and opisthosoma, tandem testes with the anterior one being asymmetrical, absence of a genital cone, a non-protrusible copulatory bursa, and ovary being pre-testicular. Herein, the intestinal region of fourteen herring gulls (70%) was infected with a diplostomidid species within the *Diplostomum* genus.

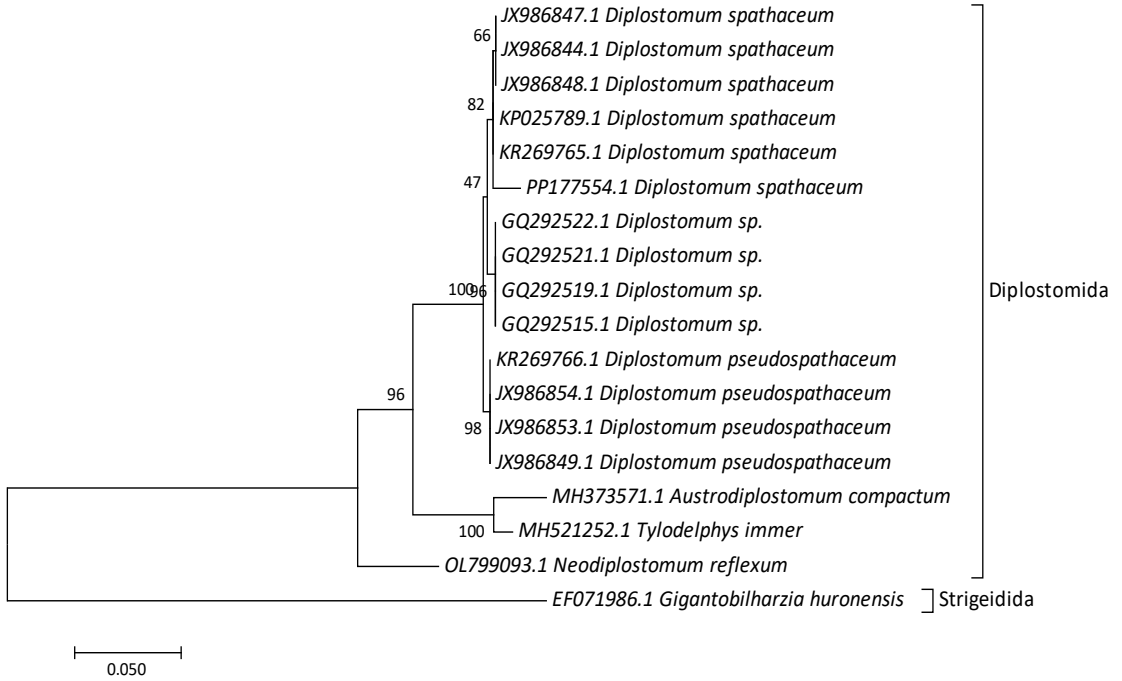

Figure 2. Molecular Phylogenetic analysis using the Maximum Likelihood method based on the Jukes-Cantor model. Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

This prevalence is similar to those mentioned previously in Yaseen and Abdullah (2018) for *D. pseudospathaceum* in *L. cachinnans* from Faw township (Southern Basrah, Iraq) (prevalence (p) = 70%). Moreover, this prevalence is lower than the previous data of *D. spathaceum* reported by Yaseen and Abdullah (2018) in *L. cachinnans* from Faw township (Southern Basrah, Iraq) (p = 100%). This prevalence is higher than the previous data of diplostomidid infection reported by Ehrhardt *et al*. (1966) in *L. atricilla* (p=59%), Threlfall (1967) in *L. argentatus* from UK (p = 0.15%), Al-Allousi (1985) in *L. ridibundus* from Baghdad (Iraq) (p = 26.5%), Mhaisen *et al*. (1990) in *L. ichinyaetus* and *L. canus* from Basrah (Iraq) (p = 47%), Tameemi (2013) in

L. genei from Basrah (Iraq) (p = 13.3%), and Pérez-del-Olmo *et al*. (2014) in *L. argentatus* and *L. ridibundus* in fish and birds from Spain (p = 60-65%).

The present diplostomidid species are completely matched with the adult stages of *D. spathaceum* described previously by Krause (1914), Dubois (1938, 1970), Cichowlas (1961), Sweeting (1976), Dick and Rosen (1981), Niewiadomska (1984), Sonin (1986), Brady (1989), Pérez-del-Olmo *et al*. (2014), Yaseen and Abdullah (2018), and Lee *et al*. (2020) with the correspondence in the morphology and dimensions of the body and internal organs, host type (*ex Larus* species), and infection-specific sites. However, there is a slight difference between the current *D. spathaceum* and those described previously by Krause (1914), Dubois (1970), Sweeting (1976), Dick and Rosen (1981), Lee *et al*. (2020) in the smaller dimensions of testes in our specimens. This is the first report on the presence of *D. spathaceum* in an avian species in Egypt.

The adults of the current *D. spathaceum* are most similar to *D. pseudospathaceum* infecting *L. argentatus* and *L. ridibundus*, which is consistent with Niewiadomska (1984). However, there are some differences between both parasite species, including: (I) the length of the pseudosuckers (smaller in *D. pseudospathaceum*), (II) the shape of the forebody (elongate or linguiform in *D. pseudospathaceum vs.* egg-shaped or oval in *D. spathaceum*), (III) the position of the ventral sucker to the holdfast organ (distant from it in *D. pseudospathaceum vs.* near to the holdfast or partly covered by it in *D. spathaceum*), (IV) the distribution of vitellaria in the forebody (strands of small follicles reaching the ventral sucker in *D. pseudospathaceum vs.* compact and rarely extend anteriorly to the holdfast organ), and (V) the shape of testes (horseshoe-shaped for posterior one in *D. pseudospathaceum*).

Moreover, the recovered *D. spathaceum* is be differentiated from other *Diplostomum* species mentioned previously in Höglund and Thulin (1992), Galazzo *et al*. (2002), Pérez-del-Olmo *et al*. (2014), Sitko and Rząd (2014), Yassen and Abdullah (2018), Lee *et al*. (2020), and Faltýnková *et al*. (2022), by the following: (I) the extent of the body division (indistinct in *D. parviventosum* in *Melanitta fusca* and *Mergus merganser*, *D. pungitii* Shigin, 1965 in *Clangula hyemalis*, *Somateria mollissima*, *Bucephala clangula*, and *Aythya fuligula*), (II) the dimensions of the body and internal organs (smaller in *D. mergi* infecting *M. merganser*, *D. parviventosum*, *D. pungitii*, *D. pusillum* from *M. merganser*, *D. phoxini* infecting *M. merganser*, *D. baeri* from *Stercoraius parasiticus*, *S. longicaudus*, *L. delawarensis*, *D. huronense*, and *D. indistinctum*), (III) the fore/hindbody ratio (fore- is longer than hind part in *D. mergi*, *D. parviventosum*, *D. pungitii*, and *D. phoxini*), (IV) the ventral/oral sucker ratio (ventral larger than oral sucker in *D. mergi*, *D. parviventosum*, *D. pungitii*, *D. pusillum*, *D. phoxini*, *D. baeri*, *D. volvens*, *D. huronense*, and *D. indistinctum*), (V) the size of pseudosuckers (larger in *D. pungitii*, *D. baeri*, and *D. volvens* from *L. ridibundus*), (VI) the distribution of vitellaria (three forward-oriented strands in *D. phoxini*), and (VIII) the size of eggs (larger in *D. mergi*, *D. parviventosum*, *D. pungitii*, *D. pusillum*, *D. phoxini*, *D. baeri*, *D. volvens*, *D. huronense*, and *D. indistinctum*).

Molecular confirmation of the recovered parasite was brought about by studying the sequence variation on the ITS1-5.8S-ITS2 gene region, which agreed with Faltýnková *et al*. (2022) showed that this nuclear genetic region is useful for the identification of the adult stages of *Diplostomum* species infecting fish-eating birds. In the present study, one sequence was obtained from the organism which clustered with the adult stages of the genus *Diplostomum* infecting *Larus* species. This is consistent with Galazzo *et al*. (2002), Pérez-del-Olmo *et al*. (2014), Brabec *et al*. (2015), and Lee *et al*. (2020) reported that the *Larus* species considered as the specific host type for the *Diplostomum* taxa. The branch that grouped members of the genus *Diplostomum* was distinct from the branch that grouped the related organisms with the genera *Austrodiplostomum*, *Tylodelphys*, and *Neodiplostomum*. In this study, all diplostomidid genera formed a monophyletic group. Our molecular findings confirmed the distinction of the organism which is identified as *D. spathaceum* from *D. pseudospathaceum*. This agreed with the previous data of Pérez-del-Olmo *et al*. (2014) studied *D. spathaceum* infecting *Larus argentatus* from Ebro Delta, Cunit (Tarragona, Spain) and confirmed its molecular identity which grouped, in one clade, with *D. pseudospathaceum* and *D. mergi*.

CONCLUSION

This study represents a first report for the morphological description of the *Diplostomum* species infecting herring gull *Larus argentatus* in Egypt, in association with the molecular data used for identification.

ACKNOWLEDGMENTS

This study was supported by the Researchers Supporting Project (RSP2024R25), King Saud University, Riyadh, Saudi Arabia.

REFERENCES

ABDEL-AAL, A.A., EL-SAYED, N.M. Internal parasites of some migrant and framed Quails in Egypt. *J. Egypt. Vet. Med. Soc. Parasitol.*, v.1, p.29-47, 2003.

ABDEL-AAL, A.A.; BADAWY, G.A.; GHATTAS, M.W. Some digenetic trematodes of the black headed gulls (*Larus ridibundus*) caught from El-Manzala Lake and Mediterranean Sea at Port Said, Egypt. *J. Egypt. Vet. Med. Assoc*., v.61, v.145-161, 2001.

ACHATZ, T.J., MARTENS, J.R., KOSTADINOVA, A. *et al*. Molecular phylogeny of *Diplostomum*, *Tylodelphys*, *Austrodiplostomum* and *Paralaria* (Digenea: Diplostomidae) necessitates systematic changes and reveals a history of evolutionary host switching events. *Int. J. Parasitol*., v.52, p.47-63, 2022.

AKSENOVA, O.V.; BESPALAYA, Y.V.; BOLOTOV, I.N.; KONDAKOV, A.V. First molecular identification of *Diplostomum spathaceum* (Rudolphi, 1819) (Trematoda: Digenea) from an intermediate host *Radix auricularia* (L., 1758) (Gastropoda: Lymnaeidae) in Russia. *Biharean Biologist*, v.10, p.90-92, 2016.

AL-ALLOUSI, J.A.A. *A survey of alimentary canal helminths of two birds: Gull (Larus ridibundus) and wood-pigeon (Columbia palambus) from Baghdadi and Baiji regions*. 1985. 124f. Thesis (Doctor) - University Baghdad.

BARATA, S.D., DÖRÜCÜ, M., SAĞLAM, N., GÜRSES, M., OTLU, ÖNDER. Molecular diversity of diplostomum spathaceum (Digenea: Diplostomidae) on the capoeta umbla and cyprinus carpio (Cypriniformes) using mitochondrial DNA barcode. *Turk. J. Fish. Aquat. Sci*., v.23, p.TRJFAS20576, 2023.

BARATA, S.D.; DORUCU, M.; GURSES. M. Identification and Molecular Investigation of Diplostomum in Capoeta umbla Caught from Freshwater Sources, Turkey. *Genet. Aquat. Organ*., v.6, p.GA454, 2022.

BLASCO-COSTA, I.; FALTÝNKOVÁ, A.; GEORGIEVA, S. *et al*. Fish pathogens near the arctic circle: molecular, morphological and ecological evidence for unexpected diversity of Diplostomum (Digenea: Diplostomidae) in Iceland. *Int. J. Parasitol*., v.44, p.703-715, 2014.

BLASCO-COSTA, I.; LOCKE, S.A. Life history, systematics and evolution of the Diplostomoidea Poirier, 1886: progress, promises and challenges emerging from molecular studies. In: ROLLINSON, D.; STOTHARD, J.R. (Eds.). *Advances in parasitology*. London: Academic Press, 2017. p.167-225.

BRABEC, J.; KOSTADINOVA, A.; SCHOLZ, T.; LITTLEWOOD, T.J. Complete mitochondrial genomes and nuclear ribosomal RNA operons of two species of *Diplostomum* (Platyhelminthes: Trematoda): a molecular resource for taxonomy and molecular epidemiology of important fish pathogens. *Parasit. Vectors*, v.8, p.336, 2015.

BRADY, A. *Studies on the Taxonomy and biology of Diplostomum species (Digenea*). 1989. These (Doctor) - Faculty of Natural Sciences,University of Stirling, Stirling, UK.

BURGER, J.; GOCHFELD, M. Family Laridae (Gulls). In: DEL HOYO, J.; ELLIOTT, A.;. SARGATAL, J. *Handbook of the birds of the world*: hoatzin to auks. Barcelona: Lynx, 1996. v.3, p.572-623.

BUSH, A.O.; LAFFERTY, K.D.; LOTZ, J.M.; SHOSTAK, A.W. Parasitology meets ecology on its own terms: Margolis, revisited. *J. Parasitol*., v.83, p.575-583, 1997.

CAVALEIRO, F.I.; PINA, S.; RUSSELL-PINTO, F. *et al*. Morphology, ultrastructure, genetics, and morphometrics of *Diplostomum* sp. (Digenea: Diplostomidae) metacercariae infecting the European flounder, *Platichthys flesus* (L.) (Teleostei: Pleuronectidae), off the northwest coast of Portugal. *Parasitol. Res*., v.110, p.81-93, 2012.

CICHOWLAS, Z. The life-cycle of Diplostomum spathaceum (Rud. 1819) in brackish waters of the Baltic Sea. *Acta Parasitol. Pol*., v.9, p.33-46, 1961.

DICK, T.A., ROSEN, R. Identification of *Diplostomum* spp. From the eyes of lake whitefish, *Coregonus clupeaformis* (Mitchell), based on experimental infection of herring gull chicks, *Larus argentatus* Pontoppidan. *Can. J. Zoo*, v.59, p.1176-1179, 1981.

DUBOIS, G. Monographie des Strigeida (Trematoda). *Mem. Soc.Neuchatel. Sci. Nat*., v.6, p.161-196, 1938.

DUBOIS, G. Synopsis des strigeidae et des diplostomatidae (Trematoda). *Mém. Soc. Neuchâte. Sci. Natu*r., v.10, p.259-727, 1970.

EHRHARDT, W.R.; HARKEMA, R.; MILLER, G.C. Trematodes from two species of gulls from North Carolina. *J. Elisha Mitchell Sci. Soc*., v.82, p.208-212, 1966.

FALTÝNKOVÁ, A.; GEORGIEVA, S.; KOSTADINOVA, A. *et al*. *Diplostomum* von Nordmann, 1832 (Digenea: Diplostomidae) in the sub-Arctic: descriptions of the larval stages of six species discovered recently in Iceland. *Syst. Parasitol*., v.89, v.195-213, 2014.

FALTÝNKOVÁ, A.; KUDLAI, O.; PANTOJA, C.; YAKOVLEVA, G.; LEBEDEVA, D. Another plea for 'best practice' in molecular approaches to trematode systematics: *Diplostomum* sp. Clade Q identified as *Diplostomum baeri* Dubois, 1937 in Europe. *Parasitology*, v.149, p.503-518, 2022.

FALTÝNKOVÁ, A.; SURES, B.; KOSTADINOVA, A. Biodiversity of trematodes in their intermediate mollusc and fish hosts in the freshwater ecosystems of Europe. *Syst. Parasitol.*, v.93, p.283-293, 2016.

GALAZZO, D.E.; DAYANANDAN, S.; MARCOGLIESE, D.J.; MCLAUGHLIN, J.D. Molecular systematics of some North American species of *Diplostomum* (Digenea) based on rDNA-sequence data and comparisons with European congeners. *Can. J. Zool.*, v.80, p.2207-2217, 2002.

GEORGIEVA, S,. SOLDÁNOVÁ, M.; PÉREZ-DEL-OLMO, A. *et al*. Molecular prospecting for European Diplostomum (Digenea: Diplostomidae) reveals cryptic diversity. *Int. J. Parasitol*., v.43, p.57-72, 2013.

GHATTAS, M.W. Investigation on enteric helminth parasites of some aquatic birds at Port Said Governorate. *J. Egypt. Med. Assoc*., v.64, p.151-175, 2004.

GORDY, M.A.; HANINGTON, P.C. A fine-scale phylogenetic assessment of digenean trematodes in central Alberta reveals we have yet to uncover their total diversity. *Ecol. Evol*., v.9, p.3153-3238, 2019.

HENEBERG, P.; SITKO, J.; TĚŠÍNSKÝ, M. Paraphyly of conodiplostomum Dubois, 1937. *Parasitol. Int.*, v.76, p.102033, 2020.

HÖGLUND, J.; THULIN, J. Identification of *Diplostomum* spp. In the retina of perch *Perca fluviatilis* and the lens of roach *Rutilus rutilus* from the Baltic Sea – an experimental study. *Syst. Parasitol*., v.21, p.1-19, 1992.

HOOGENDOORN, C.; SMIT, N.J.; KUDLAI, O. Resolution of the identity of three species of Diplostomum (Digenea: Diplostomidae) parasitising freshwater fishes in South Africa, combining molecular and morphological evidence. *Int. J. Parasitol. Parasit. Wildl*. v.11, p.50-61, 2020.

KARVONEN, A. *Diplostomum spathaceum* and related species. In: WOO, P.T.K.; BUCHMANN, K. (Eds.). *Fish parasites*: pathobiology and protection. Cambridge: CAB International, 2012. p.260-269.

KARVONEN, A.; TERHO, P.; SEPPÄLÄ, O.; JOKELA, J.; VALTONEN, E.T. Ecological divergence of closely related *Diplostomum* (Trematoda) parasites. *Parasitology*, v.133, p.229-235, 2006.

KHOSHNAW, L.S.H.; ABDULLAH, S.M.A. Ultramorphology and molecular studies of *Diplostomum paracaudum* (Trematoda) collected in greater Zab River from Kurdistan region, Iraq. *Appl. Ecol. Environ. Res*., v.21, p.5163-5173, 2023.

KRAUSE, R. Beitrag zur kenntnis der hemistominen. *Z. Wissenschaftl. Zool*., v.112, p.93-238, 1914.

KUDLAI, O.; OROS, M.; KOSTADINOVA, A.; GEORGIEVA, S. Exploring the diversity of Diplostomum (Digenea: Diplostomidae) in fishes from the river Danube using DNA mitochondrial barcodes. *Parasit. Vectors*, v.10, p.592, 2017.

KUMAR, S.; STECHER, G.; LI, M.; KNYAZ, C.; TAMURA, K. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol*., v.35, p.1547-1549, 2018.

LEE, Y.I.I.; SEO, M.; CHAI, J.Y. Intestinal flukes recovered from a herring gull, *Larus argentatus*, in the Republic of Korea. *Korean J. Parasitol*., v.58, p.81-86, 2020.

LOCKE, S.A.; AL-NASIRI, F.S.; CAFFARA, M. *et al*. Diversity, specificity and speciation in larval Diplostomidae (Platyhelminthes: Digenea) in the eyes of freshwater fish, as revealed by DNA barcodes. *Int. J. Parasitol*., v.45, p.841-855, 2015.

LOCKE, S.A.; DRAGO, F.B.; NÚÑEZ, V.; SOUZA, G.T.R.E.; TAKEMOTO, R.M. Phylogenetic position of Diplostomum spp. from new world herons based on complete mitogenomes, rDNA operons, and DNA barcodes, including a new species with partially elucidated life cycle. *Parasitol. Res*., v.119, p.2129-2137, 2020.

MARCOGLIESE, D.J.; DUMONT, P.; GENDRON, A.D. *et al*. Spatial and temporal variation in abundance of *Diplostomum* spp. In walleye (*Stizostedion vitreum*) and white suckers (*Catostomus commersoni*) from the St. Lawrence River. *Can. J. Zool.*, v.79, p.355-369, 2001.

MHAISEN, F.T.; KHAMEES, N.R.; AL-SAYAB, A.A. Flat worms (Platyhelminthes) of two species of gull (*Larus ichthyaetus* and *L. canus*) from Basrah. *Zool. Middle East*, v.4, p.113-116, 1990.

NDEDA, V.M.; OWITI, D.O.; AKETCH, B.O.; ONYANGO, D.M. Genetic relatedness of

Diplostomum species (Digenea: Diplostomidae) infesting Nile Tilapia (*Oreochromis niloticus* L.) in Western Kenya. *Open J. Appl. Sci.*, v.3, p.441-448, 2013.

NIEWIADOMSKA, K. [*Trematoda, the general part; systematic part - aspidogastrea, digenea*: strigeida], Fauna Słodkowodna Polski, 34 A, Polish Hydrobiological Society. [Polish]: University of Łódź, Łódź, 2010. 388p.

NIEWIADOMSKA, K. Present status of *Diplostomum spathaceum* (Rudolphi, 1819) and differentiation of *Diplostomum pseudospathaceum* nom. nov. (Trematoda: Diplostomidae). *Syst. Parasitol*., v.6, p.81-86, 1984.

NIEWIADOMSKA, K.; LASKOWSKI, Z. Systematic relationships among six species of *Diplostomum* Nordmann, 1832 (Digenea) based on morphological and molecular data. *Acta Parasitol*., v.47, p.20-28, 2002.

PÉREZ-DEL-OLMO, A., GEORGIEVA, S., PULA, H.J., KOSTADINOVA, A. Molecular and morphological evidence for three species of *Diplostomum* (Digenea: Diplostomidae), parasites of fishes and fish-eating birds in Spain. *Parasit. Vector*, v.7, p.502, 2014.

POIRIER, J. Sur les diplostomidae. *Arch. Zool. Exp. Gen.*, v.2, p.327-346, 1886.

RUDOLPHI, K.A. Entozoorum synopsis cui accedunt mantissa duplex et indices locupletissimi/auctore, Carolo Asmund Rudolphi. 1819. Available in: https://www.biodiversitylibrary.org/page/9698017. Accessed in: 1, April, 2019.

SELBACH, C.; SOLDÁNOVÁ, M.; GEORGIEVA, S.; KOSTADINOVA, A. SURES, B. Integrative taxonomic approach to the cryptic diversity of Diplostomum spp. in lymnaeid snails from Europe with a focus on the 'Diplostomum mergi' species complex. *Parasit. Vectors*, v.8, p.300, 2015.

SEPPÄLÄ, O.; LILJEROOS, K.; KARVONEN, A.; JOKELA, J. Host condition as a constraint for parasite reproduction. Oikos, v.117, p.749-753, 2008.

SHIGIN, A.A. Some results of the study of the systematics of metacercariae of Diplostomum – the pathogens causing diplostomosis of freshwater fishes of the USSR. *Mater. Nauk. Konf*., v.1, p.261-265, 1965.

SITKO, J.; RZĄD, I. *Diplostomum* and *Ornithodiplostomum scardinii* (Diplostomidae, Digenea) species from naturally infected birds (Anatinae) in the Czech Republic and in Poland: morphological, morphometric and ecological features. *Helminthologia*, v.51 p.215-224, 2014.

SOKOLOV, S.G.; IESHKO, E.P.; LEBEDEVA, D.I. Resurrection of *Diplostomum numericum* Niewiadomska, 1988 (Digenea, Diplostomatoidea: Diplostomidae) based on novel molecular data from the Type-Host. *Diversity*, v.15, p.840, 2023.

SOLDÁNOVÁ, M., GEORGIEVA, S., ROHÁČOVÁ, J. *et al*. Molecular analyses reveal high species diversity of trematodes in a sub-Arctic lake. *Int. J. Parasitol*., v.47, p.327-345, 2017.

SONIN, M.D. *A key of the trematoda of piscivorous birds within the Palaearctic area*. Moscow: Nauka, 1986. p.127-145.

SWEETING, R.A. An experimental demonstration of the life cycle of a *Diplostomum* from *Lampetra fluviatilis* Linnaeus, 1758. Z. *Parasitenkd*., v.49, p.233-242, 1976.

TADROS, S.W.; ABO-ESA, J.F.K.; EL-GAYAR, A.K. Some studies on digenetic trematodes of herring gull (*Larus argentatus*) at Port Said governorate. *Assiut Vet. Med. J*., v.59, p.161-169, 2013.

TAMEEMI, I.A.A. *Helminthes parasitized on some aquatic birds and the importance of insects in the life cycle of some of them in Basrah province*. 2013. 241f. Thesis (Doctor) - College of Science, University of Basrah, IRQ.

THRELFALL, W. Diseases and Pathological conditions of the herring gull (*Larus argentatus* Pontopp.) excluding helminth infestations. *Bull. Wildl. Dis. Assoc*., v.3, p.62-67, 1967.

VIVAS MUÑOZ, J.C.; FELD, C.K.; HILT, S. *et al*. Eye fluke infection changes diet composition in juvenile European perch (*Perca fluviatilis*). *Sci. Rep*., v.11, p.3440, 2021.

VON NORDMANN, A.V. *Mikrographische Beitriige zur Naturgeschichte der wirbellosen thiere*. Marburg: Philipps-Universität Marburg, 1832. p.28-49.

YASEEN, A.M.; ABDULLAH, B.H. First record of three species of trematode in Caspin Gull (*Larus cachinnans* Pallas, 1811) in Faw township, southern of Basrah, Iraq. *Basrah J. Sci*., v.36, p.53-67, 2018.