Egyptian Journal of Animal Health

P-ISSN: 2735-4938 On Line-ISSN: 2735-4946
Journal homepage: https://ejah.journals.ekb.eg/

Prevalence and Molecular Characterization of some Heterophyid species in Stray Cats in Alexandria

Neveen, S. Satour* and Rehab, R. Abd ELMaged**

*Parasitology Department, Animal Health Research Institute, Alexandria Lab.
**Parasitology Department, Animal Health Research Institute, Mansoura Lab.
Agriculture Research Center (ARC), Egypt.

Received in 17/7/2024
Received in revised from 7/8/2024
Accepted in 26/8/2024

Keywords:
Heterophyid sp,
Heterophyes heterophyes
Cats
PC
ITS2
Egypt

ABSTRACT

Stray cats are highly susceptible to a variety of parasitic infections because they have the habit of roaming freely. A total of 50 stray cats were collected from various areas in Alexandria governorate. The results indicated that 17 (34%) stray cats were infected with heterophyid species. The identified heterophyid species were *Heterophyes heterophyes* (16%) then *Haplorchis taichue* (8%), *Pygidiopsis geneta* (6%), *Ascocotyle longa* (4%), *Stictodora sawakinsis* (4%) and *Procerovum varium* (2%). DNA sequencing of 539 bp of ITS2 gene was generated. As shown in sequence distance figure, the sequenced strains showed 99.5 - 100% identity to *H. heterophyes* strains confirming the clustering of the study strain with *H. heterophyes*. The study revealed that stray cats are a reliable indicator of fish -borne heterophyid species in the environment given that their relevance of public health must be carefully considered.

INTRODUCTION:

The most popular pet worldwide is the cat. Cats are obligate carnivores with different breeds, colors and classified into domestic cats and wild cats. Stray cats act as potential reservoir hosts of a wide range of helminth parasites, particularly digenetic trematodes, reflecting both veterinary and medical importance **(Millan and Casanova, 2009; El-Dakhly et al. 2017).**

Heterophyids are a group of trematodes that are very small (1–2 mm in length) infect vertebrate animals, including mammals and birds **(Yamaguti, 1958)**. This family contains at least 36 genera, 13 of which are known to be zoonotic **(Chai, 2007)** including *Metagonimus,Heteorphes, Haplorchis, Pygidiopsis, Heterophyopsis, Stellantchasmus, Centrocestus, Stictodora, Procerovum, Acanthotrema, Apophallus, Ascocotyle and Cryptocotyle. They are exclusively fishborne and contracted to humans by ingesting* uncooked or poorly prepared freshwater or brackish water fish **(Chai, 2007 and Chai, 2014).**

In 1851, during the autopsy of an Egyptian cat in Cairo, Bilharz found *Heterophyes heterophyes* (**Chai, 2007**). It causes human infec-

Corresponding author: **Neveen, S. Satour**, Parasitology Department, Animal Health Research Institute, Alexandria Lab., Agriculture Research Center (ARC), Egypt.
E-mail: neveen.satour@yahoo.com
DOI: 10.21608/ejah.2024.386208

tions along Nile Delta of Egypt and Sudan, Middle East, southeastern Europe and India (**Yu and Mott, 1994; Mahanta et al. 1995 and Pica et al. 2003**).

Due to the small size of the adult stage and the considerable morphological similarity between closely related species of heterophyid flukes, morphological study might be insufficient for accurate species identification (**Henedi, 2019**). The availability of molecular study has allowed the accurate identification using the second internal transcribed spacer (ITS2) region. This region has been successfully used to genetically identify several *Heterophyidae* intestinal flukes (**Masala et al. 2016**).

So, the current study aims to determine the prevalence, morphological and molecular identification of *Heterophyes heterophyes* in stray cats. It also aims to assess the prevalence of some other heterophyid species.

MATERIALS and METHODS

Ethical considerations:

To prevent harm and minimize the pain, all procedures were created in accordance with national laws and regulations regarding handling of animals.

Study area:

The capital of Alexandria Province, Alexandria City (31°12′N29°55′E), is located 114 miles (183 kilometers) northwest of Cairo along the Mediterranean Sea coast in north-central Egypt. **(Frihy et al. 1996 and Elseify et al. 2017).**

Sample collection:

A total number of 50 stray cats were surveyed for the presence of heterophyid species during the period from January to September 2023 from different areas in Alexandria governorate. The trapped cats were transferred to the laboratory of Parasitology Department of Animal Health Research Institute; Alexandria lab.

The trapped cats were humanely euthanized by Xylaject (Xylazine Hydrochloride 23.3 mg, Adwia company) with intramuscular injection (0.5 ml\10 kg) then humanely sacrificed by overdose of chloroform and the age was assessed by dentition according to **(Floyd, 1991).**

Necropsy of Cats

During necropsy, the abdominal cavity was opened, the gastrointestinal tract was removed and then it was longitudinally opened and washed in 0.85% saline. The mucosa was scraped. The epithelial scrapings were passed through 60-80 mesh wire sieves. The contents of the sieves were washed with tap water and sediments were allowed to settle down and carefully examined under a stereomicroscope to discover minute parasites **(Soulsby, 1982 and El-Dakhly et al., 2012).**

Preparation of trematodes for examination

The collected trematode worms were washed in normal saline, relaxed in refrigerator, fixed in 10% formalin, stained using acetic acid-alum carmine with concentration 1%, dehydrated via serial passage of ethyl alcohol, cleared in clove oil, washed momentarily in xylene and were mounted in Canada balsam **(Kruse and Pritchard 1982).**

Identification of adult worms was done according to the key given by **Yamaguti (1958) and El-Shahawi (1983).**

Material for DNA extraction: The collected *Heterophyes* worms were fixed in 96% ethanol until prepared for DNA extraction.

DNA extraction. DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 25 mg of the sample was incubated with 20 µl of proteinase K and 180 µl of ATL buffer at 56^{O}C overnight. After incubation, 200 µl of AL buffer was added to the lysate, incubated for 10 min. at 72^{O}C, then 200 µl of 100% ethanol was added to the lysate. The lysate was then transferred to silica column and centrifugated. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in

the kit.

Oligonucleotide Primer: Primers used were supplied from **Metabion (Germany)** are listed in table (1).

PCR amplification: Primers were utilized in a 25- µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 5.5 µl of water and 5 µl of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler.

Analysis of the PCR Products: The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products was loaded in each gel slot. Generuler 100 bp ladder (Fermentas, Germany) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Sequencing and phylogenetic analysis: PCR products were purified using QIAquick PCR Product extraction kit (Qiagen, Valencia). Bigdye Terminator V3.1 cycle sequencing kit (Perkin-Elmer) was used for the sequence reaction and then it was purified using Centrisep spin column. DNA sequences were obtained by Applied Biosystems3130 genetic analyzer (HITACHI, Japan), a BLAST® analysis (Basic Local Alignment Search Tool) **(Altschul et al. 1990)** was initially performed to establish sequence identity to GenBank accessions. The phylogenetic tree was created by the MegAlign module of LasergeneDNAStar version 12.1 **Thompson et al. (1994)** and phylogenetic analyses was done using maximum likelihood, neighbour joining and maximum parsimony in MEGA6 **(Tamura et al. 2013).**

Table 1. Primers sequences, target genes, amplicon sizes and cycling conditions.

Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
Trematode ITS2	GGTACCGGTGGATCACTCGGCTCGTG GGGATCCTGGTTAGTTTCTTTTCCTCCGC	539	94˚C 5 min.	94˚C 30 sec.	57˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Arya et al. (2016)**

RESULTS

In the current study, 17 out of 50 stray cats were infected with heterophyid species representing (34%). The recovered heterophyid species were *Heterophyes heterophyes* (16%), *Haplorchis taichui* (8%), *Pygidiopsis geneta* (6%), *Ascocotyle longa* (4%), *Stictodora sawakinsis* (4%) and *Procerovum varium* (2%) (table 2).

Table 2. Prevalence of heterophyid species in stray cats (n = 50):

Heterophyid species	Infected cats	%
Heterophyes heterophyes	8 (6 S + 2 M)	16
Haplorchis taichui	4 (2 S + 2 M)	8
Pygidiopsis geneta	3 (2 S + 1 M)	6
Ascocotyle longa	2 (1 S + 1 M)	4
Stictodora sawakinsis	2	4
Procerovum varium	1	2
Total	17	34

S: Single infection, M: Mixed infection
N.B.: One cat had a mixed infection of *Heterophyes heterophyes* and *Haplorchis taichui*
One cat had a mixed infection of *Heterophyes heterophyes* and *Pygidiopsis geneta*
One cat had a mixed infection of *Haplorchis taichui* and *Ascocotyle longa*

Table (3) showed that the prevalence of heterophyid species according to age were 25% in young age ≤1 years old and 40% in adult age > 1 years old. The prevalence was higher in females (38.5%) than males (29.2%).

Table 3. Prevalence of heterophyid species in stray cats according to age and gender (n = 50):

		Examined cats	Infected cats	%
Age	≤ 1 year	20	5	25
	> 1 year	30	12	40
Gender	Female	26	10	38.5
	Male	24	7	29.2

Morphological identification of heterophyid species: -

***Heterophyes heterophyes*:**

Short pear-shaped flukes measured 1-1.5 long x 0.3-0.4 mm at their greatest width, flattened dorsoventrally, rounded at both extremities, with spiny integument. The oral sucker measured approximately 50 µm in diameter. The oesophagus measured 0.2 mm long. The ventral sucker was well developed and located at middle of the body between 2 ceca, the genital sucker was oval, armed with incomplete circle of 70-80 spines, lying beside ventral sucker. The testes were oval in shape, lying side-by-side near the posterior extremity of the body and measured about 50 µm. The ovary was round in shape, lying on the midline in front of the testes. The uterus filled the space between the ventral suckers and the testes (Fig. 1A).

***Haplorchis taichui*:**

Spiny elongated flukes measured 0.7-0.8 x 0.2-0.3 mm. It has terminal oral sucker. The ventral sucker has a semi-lunar group of 12–16 long, crescentic and hollow spines. It has single, fan shaped, lobulated testis and located at the posterior part of the body. The ovary lies pretesticular. The uterus contains numerous eggs extends from behind the bifurcation of intestinal caeca to the posterior end (Fig. 1B).

***Pygidiopsis geneta*:**

Spiny flattened flukes measured 0.4-0.7 x 0.2-0.4 mm, the oral sucker is spiny and sub terminal, the two testes are symmetrical and located at the posterior end of the body (Fig. 1C).

***Ascocotyle longa*:**

A small elongated conical-shaped flukes measured 0.6-0.65 x 0.18-0.2 mm with cone shape prolongation at oral sucker and surround with minute spines. The hind part of the body is wider than the fore one. The ventral sucker centrally located between the bifurcation of two ceca. The genital opening is just anterior to the ventral sucker. The majority of the reproductive organs are located posterior to the

ventral sucker. Paired testes are symmetrical and located at the posterior end of the body (Fig. 1D).

***Stictodora sawakinsis*:**

A small elongated pyriform flukes measured 0.8-0.9 x 0.38-0.4. The ventrogenital sac and a gonotyl armed with spines in the form of a comma or reversed comma lying along their lateral margin. Seminal vesicle is bilobed and seminal receptacles well developed found at the area between the two testes. The testes are obliquely oval to round in shape and located at the second third of the body. The ovary is located between the two testes (Fig. 1E).

***Procerovum varium*:**

A small pear-shaped spiny flukes measured 0.35-0.4 × 0.2-0.25 mm with the greatest width at the posterior third of the body. The oral sucker is sub terminal. The ventral sucker is very small, located just behind the bifurcation of intestinal ceca. Intestinal ceca bifurcate at the level of anterior third and terminate at the posterior fourth of the fluke. The ovary is spherical and located sagital to the ventral sucker. The testes are sub globular-shaped and located at the middle of the hind body (Fig. 1F).

Sequencing and genotyping of isolates: The ITS2 gene of the trematode species was amplified and yielded the expected PCR product size (539 bp) from samples (Fig. 2), then published (GenBank accession number OR509796). As shown in sequence distance figure, the sequenced strains showed 99.5 - 100% identity to *H. heterophyes* strains confirming the clustering of the study strain with *H. heterophyes*. The closest identities to other strains were as follow; 94.4 - 100% (*H. dispar*), 80.6 - 100% (*H. nocens*) (Fig 3). Phylogenetic tree cleared the clustering of the collected *H. heterophyes* with *H. heterophyes* strains (Fig.4).

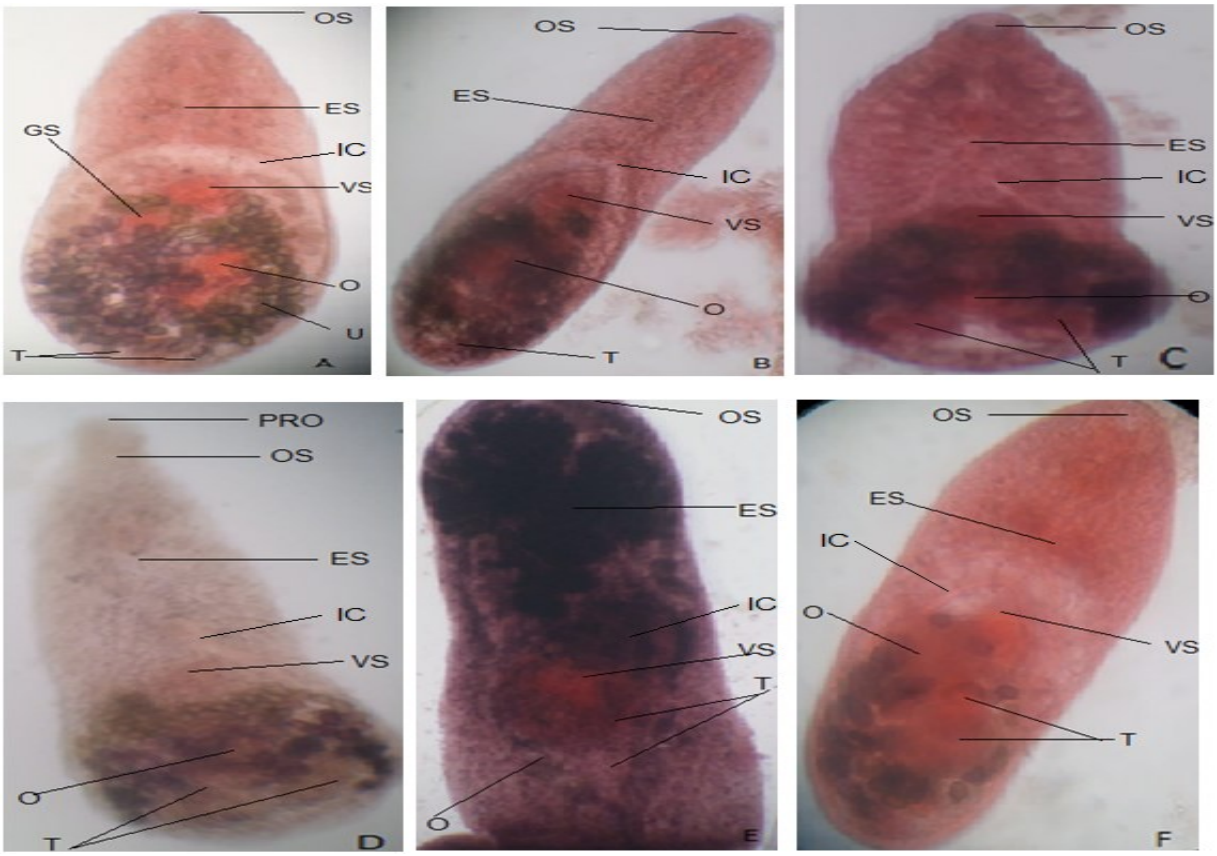

Fig. (1): A- Heterophyes heterophyes (x10). B- *Haplorchis taichui* (x10). C- *Pygidiopsis geneta* (x10). D- *Ascocotyle longa* (x10). E- *Stictodora sawakinsis* (x10). F- *Procerovum varium* (x10). (OS = oral sucker; VS = ventral sucker; GS = genital sucker; T = testis; O = ovary; ES = esophagus; U = uterus; PRO = cone shape prolongation; IC = intestinal ceca).

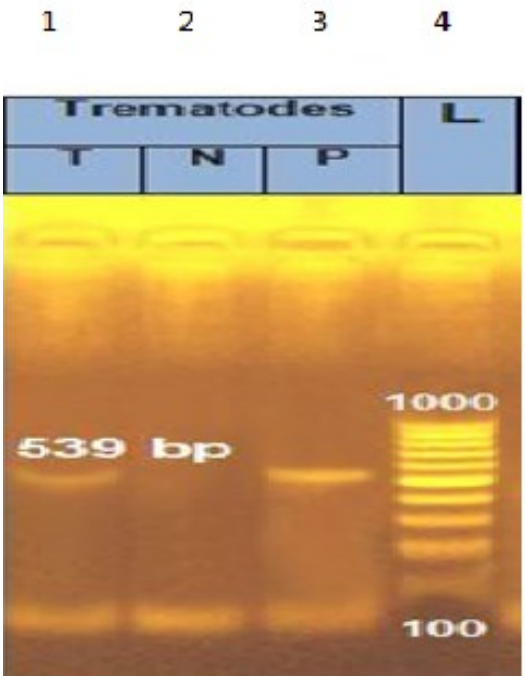

Fig. (2) PCR results for trematode ITS2 gene showing positive amplification of 539 bp of ITS2 gene in tested sample. L [Gene ruler 100 bp ladder (Fermentas, thermo 100-1000 bp)].
Lane 1: Sample Lane 2: Negative control Lane 3: Positive control Lane 4: 100-1000 bp. ladder

Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23		
1		99.7	100.0	100.0	100.0	99.7	99.5	100.0	96.9	96.9	95.0	95.0	80.1	80.1	79.6	78.8	79.6	78.5	79.3	76.2	77.2	74.6	75.9	1	KU674951 Heterophyes heterophyes He1
2	0.3		99.7	99.7	99.7	99.7	99.5	99.7	96.6	96.6	94.8	94.8	80.1	80.1	79.6	78.8	79.3	78.5	79.6	76.2	77.2	74.3	75.7	2	MW131526 Heterophyes heterophyes Hetro1
3	0.0	0.3		100.0	100.0	99.7	99.5	100.0	96.9	96.9	95.0	95.0	80.1	80.1	79.6	78.8	79.6	78.5	79.3	76.2	77.2	74.6	75.9	3	KX431325 Heterophyes heterophyes KWHH02
4	0.0	0.3	0.0		100.0	99.7	99.5	100.0	96.9	96.9	95.0	95.0	80.1	80.1	79.6	78.8	79.6	78.5	79.3	76.2	77.2	74.6	75.9	4	KX431324 Heterophyes heterophyes KWHH23
5	0.0	0.3	0.0	0.0		99.7	99.5	100.0	96.9	96.9	95.0	95.0	80.1	80.1	79.6	78.8	79.6	78.5	79.3	76.2	77.2	74.6	75.9	5	OP750427 Heterophyes heterophyes Bori-1
6	0.3	0.3	0.3	0.3	0.3		99.5	99.7	96.6	96.6	94.8	94.8	80.1	80.1	79.6	78.8	79.3	78.5	79.3	76.2	77.2	74.3	75.7	6	OP750428 Heterophyes heterophyes Bolti1
7	0.0	0.0	0.0	0.0	0.0	0.0		99.5	96.3	96.3	94.5	94.5	79.8	79.8	79.3	78.5	79.1	78.3	79.1	75.9	77.0	74.1	75.4	7	OM439579 Heterophyes heterophyes HH201
8	0.0	0.3	0.0	0.0	0.0	0.3	0.0		96.9	96.9	95.0	95.0	80.1	80.1	79.6	78.8	79.6	78.5	79.3	76.2	77.2	74.6	75.9	8	OR509796 Heterophyes heterophyes Alex
9	3.5	3.8	3.5	3.5	3.5	3.8	3.5	3.5		100.0	94.5	94.5	81.2	80.9	80.6	79.3	80.4	79.1	79.8	76.2	78.0	74.9	75.7	9	KX431328 Heterophyes dispar KWHD23
10	3.5	3.8	3.5	3.5	3.5	3.8	3.5	3.5	0.0		94.5	94.5	81.2	80.9	80.6	79.3	80.4	79.1	79.8	76.2	78.0	74.9	75.7	10	KX431327 Heterophyes dispar KWHD29
11	4.8	5.1	4.8	4.8	4.8	5.1	4.8	4.8	5.4	5.4		100.0	80.6	81.4	80.1	80.1	79.3	79.1	78.5	76.2	78.0	75.7	77.2	11	KU674960 Heterophyes nocens H.noc
12	4.8	5.1	4.8	4.8	4.8	5.1	4.8	4.8	5.4	5.4	0.0		80.6	81.4	80.1	80.1	79.3	79.1	78.5	76.2	78.0	75.7	77.2	12	KU674959 Heterophyes nocens Hno66
13	18.7	18.7	18.7	18.7	18.7	18.7	18.4	18.7	17.2	17.2	17.7	17.7		93.7	99.0	92.9	86.1	83.2	86.6	82.7	90.8	81.9	81.9	13	MF438060 Apophallus donicus AV2
14	19.0	19.1	19.0	19.0	19.0	19.0	18.7	19.0	17.9	17.9	17.0	17.0	6.8		93.5	96.3	85.6	82.7	86.6	83.0	89.8	82.2	82.2	14	MZ595816 Cryptocotyle lingua ITS-34
15	19.5	19.5	19.5	19.5	19.5	19.5	19.2	19.5	17.9	17.9	18.5	18.5	1.1	7.2		92.1	85.3	82.7	86.1	82.5	90.8	81.7	82.2	15	MH025623 Cryptocotyle lata 17.2
16	20.6	20.6	20.6	20.6	20.6	20.6	20.3	20.6	19.8	19.8	18.5	18.5	7.4	3.8	8.4		83.8	82.2	85.1	81.4	88.0	80.9	80.4	16	MF438074 Apophallus muehlingi RK2
17	21.8	22.2	21.8	21.8	21.8	22.2	21.9	21.8	20.6	20.6	21.3	21.3	13.9	14.9	15.0	17.0		83.2	90.1	80.1	84.3	80.1	79.6	17	KT883855 Pholeter gastrophilus 924
18	19.3	19.3	19.3	19.3	19.3	19.3	19.0	19.3	18.5	18.5	17.6	17.6	14.6	15.7	15.3	16.0	16.9		82.5	77.0	82.7	77.0	77.7	18	EU826639 Procerovum sp. 2 KB-2008
19	21.8	21.4	21.8	21.8	21.8	21.8	21.5	21.8	21.0	21.0	22.0	22.0	12.8	13.1	13.5	14.8	11.2	17.6		80.9	84.8	79.6	78.5	19	MF978378 Ascocotyle pindoramensis E516
20	23.3	23.4	23.3	23.3	23.3	23.3	23.0	23.3	23.3	23.3	23.2	23.2	15.0	15.1	15.4	16.9	21.0	22.5	19.5		80.9	89.0	90.1	20	EF116639 Retrovarium valdeparvum
21	22.9	22.9	22.9	22.9	22.9	22.9	22.6	22.9	21.6	21.6	20.7	20.7	8.1	9.0	8.2	11.0	16.8	15.8	15.8	18.1		81.7	80.9	21	MT231323 Metorchis orientalis MOBS
22	23.3	23.7	23.3	23.3	23.3	23.8	23.4	23.3	22.9	22.9	22.2	22.2	15.4	15.4	15.8	16.7	19.5	22.2	19.9	10.2	15.6		92.1	22	FJ154899 Lobosorchis tibaldiae
23	22.2	22.6	22.2	22.2	22.2	22.6	22.3	22.2	22.6	22.6	20.7	20.7	16.8	16.8	16.4	19.0	21.1	21.9	22.3	9.6	17.4	7.6		23	EU571256 Siphoderina infirma
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23		

Fig. (3) Sequence distance of the ITS2 gene of the tested trematode strain (generated by lasergene software) showing identity range of 99.5-100% with *Heterophyes heterophyes* strains.

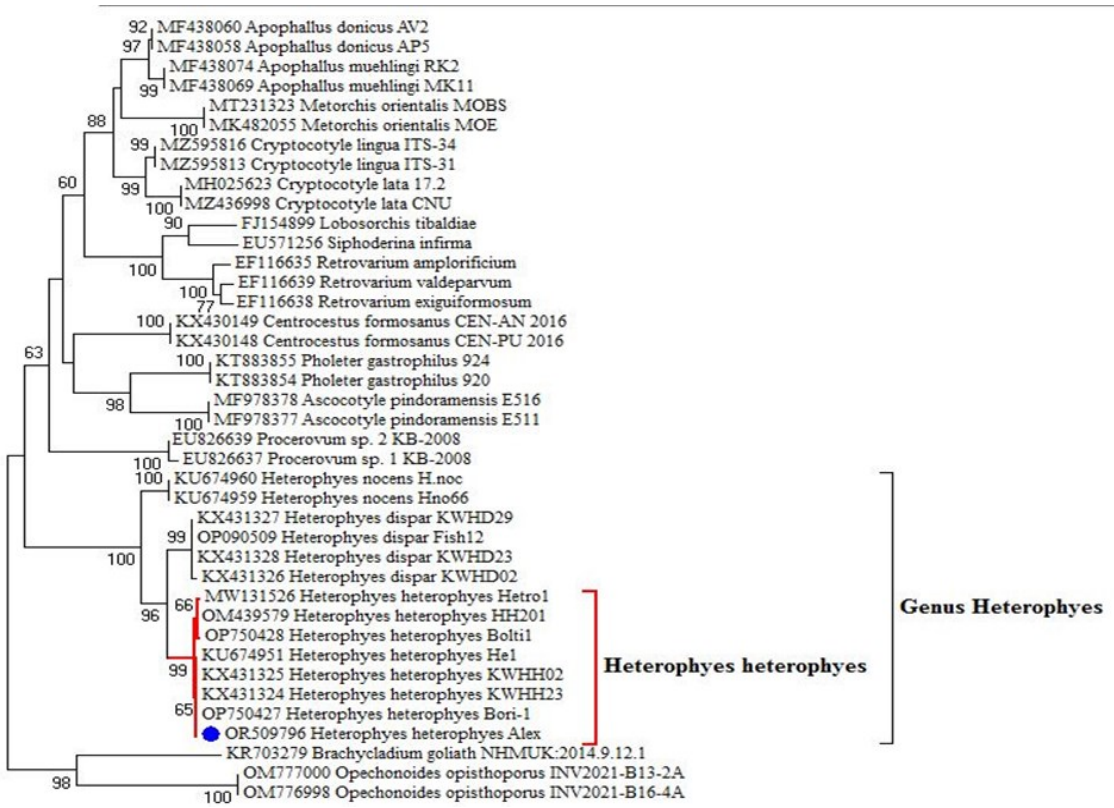

Fig. (4) Phylogenetic relatedness of the trematode ITS2 gene. Maximum – likelihood unrooted tree generated after 500 bootstraps indicated clustering of the tested strain with *Heterophyes heterophyes* strains apart from other trematodes

DISCUSSION

Felids, particularly cats, harbor many species of digenetic trematodes and act as final hosts of these trematodes. Most of them are with zoonotic importance, especially in low-income countries **(Arafa et al. 1978)**.

Chai and Lee (2002) recorded that all trematodes found in their study were transmitted by eating raw or undercooked fish and most of them had been reported to cause health problems in humans**.** They can lead to erratic parasitism in humans and their eggs may be found within inflammatory reactions of affected organs, which can lead to further complications when eggs are released into the blood stream through the intestinal wall **(Chai et al. 1986).**

In the present study the prevalence of heterophyid species from stray cats was 34% in Alexandria governorate. Higher prevalence was recorded by **Shin et al. (2015)** in Korea (55.5%). On the other hand, lower prevalence was reported by **El-Azazy et al. (2015)** in Kuwait (24.6%), **Marey (2016)** in Egypt (24.3%) and **El-Dakhly et al. (2020)** in Egypt (6.38%). It seems that fish are the primary diet of stray cats in Alexandria governorate. This is due to being a coastal country and their people diets mainly depend on seafood, that proving high prevalence of heterophyid species as recorded in this study.

The reported heterophyid species were of *Heterophyes heterophyes* (16%) which was the most abundant heterophyid species in the present study, then *Haplorchis taichue* (8%), *Pygidiopsis geneta* (6%), *Ascocotyle longa* (4%), *Stictodora sawakinsis* (4%) and *Procerovum varium* (2%). Nearly coinciding with such finding, **El-Azazy et al. (2015)** reported *Heterophyes heterophyes* (15.8%), *Haplorchis taichue* (3.8%) and *Stictodora sawakinsis* (2.1%). **El-Dakhly et al. (2017)** recorded *Het-*

erophyes heterophyes (3.2%), *Haplorchis* species (1.6%), *Pygidiopsis summa* (1.6%), *Procerovum varium* (1.6%) and *Ascocotyle* species (1.6%) and **El-Dakhly et al. (2020)** found *Heterophyes heterophyes* (6.38%), *Pygidiopsis summa* (2.13%), *Procerovum varium* (2.13%) and *Ascocotyle* species (2.13%). *Heterophyes heterophyes* infection is common in Egypt, where the pickled mullet (Mugil cephalus) is traditionally eaten at the feast of Sham- al Nessim (**Woo, 2006**). In **1933, Khalil** reported that encysted metacercaiae of *Heterophyes heterophyes* could remain in salted fish (locally known as Fessikh) and remain viable for at least week.

The present investigation revealed higher prevalence rate of heterophyid species in adult age > 1 years old (40%) than in young age ≤ 1 years old (25%). The result was nearly agreed with the previous studies obtained by **El-Azazy et al. (2015)** who recorded higher prevalence in adult (28.7%) than in Juvenile (16.2%). **Marey (2016)** reported higher prevalence in adult age > 1 years old (40.7%) than in young age ≤1 years old (31.3%). **El-Dakhly et al. (2017)** reported the highest infection rates in cats aged more than 3 years (9.68%). The higher prevalence observed in adult cats indicates longer exposure and accumulation of parasites **(Dung et al. 2007).** Additionally, it is related to competition for food, as adults are better able than younger cats to obtain fish offal from fish markets.

In the present study, the prevalence of heterophyid species was higher in females (38.5%) than in males (29.2%). This was agreed with the earlier report by **El-Azazy et al. (2015) and El-Dakhly et al. (2017)** who recorded higher prevalence in females than in males but disagreed with **Marey (2016)** who reported higher incidence in males than in females. Meanwhile, **Thabit (2011)** elucidated that the infection rates among cats are not related to the gender.

Genus *Heterophyes*, a total of 18 species or subspecies had been recognized **(Chai, 2014).** However, three species were moved to another genus *Alloheterophyes* by **Pearson (1999)** and nine species were synonymized with *H. heterophyes* or other pre-existing species **(Chai, 2014)**. Only six Heterophyes species are currently recognized as legitimate **(Pearson, 1999 and Chai, 2014)**, namely, *H. heterophyes*, *H. nocens*, *H. dispar*, *H. aequalis*, *H. indica* and *H. pleomorphis*. Four species, *H. heterophyes*, *H. nocens*, *H. dispar*, and *H. aequalis* are known to infect humans **(Ashford and Crewe, 2003 and Chai, 2014).**

The morphological characters of *H. heterophyes* was agreed with **Yamaguti (1958); El-Azazy et al. (2015)** and **El-Dakhly et al. (2020).**

The results of gene sequence analysis were in agreement with morphological data, indicating that sequencing of the Internal Transcribed Spacer 2 (ITS2) could be usefulness in differentiating *H. heterophyes* from other members of family *Heterophyidae* (GenBank accession number OR509796). ITS2 and 28S rDNA regions showed high levels of intraspecific divergence within the family *Heterophyidae* **(Masala et al. 2016; Henedi, 2019 and Attia et al. 2021)**. The 28S gene, although it has been often used for inter-species analyses of *Heterophyidae* and *Opisthorchiidae* but characterised by a lower level of genetic variation so, considered less suitable for the analysis within the family *Heterophyidae*. The deeper level of resolution obtained for the ITS2 region suggests this marker as a valuable tool for the molecular taxonomy within the family *Heterophyidae*, **(Lee et al. 2004 and Thaenkham et al. 2011).**

CONCLUSION

This study demonstrated that stray cats are reliable indicators of zoonotic heterophyids. The high prevalence of *H. heterophyes* emphasizes the need to raise public awareness about the zoonotic significance of this intestinal fluke. Parasitologists and veterinarians should educate people about the importance of snails and fish as intermediate hosts for digenetic trematodes as well as the hygienic disposal of these hosts to reduce the possibility of completing the life cycles of the flukes. The sequencing of ITS2 region proved their usefulness in differentiating *H. heterophyes* from other members of family *Heterophyidae*.

As the members of family Heterophyidae, more

than 10 species, i.e., Metagonimus spp., Pygidiopsis summa, Heterophyes nocens, Stellantchasmus falcatus, Heterophyopsis continua, Acanthotrema felis, Centrocestus armatus, Procerovum varium, Cryptocotyle concava, and Sticto

REFERENCES

Altschul SF, Gish W, Miller W, Myers EW and Lipmanl DJ. 1990. Basic Local Alignment Search Tool. J Mol Biol (215): 403-410.

Arafa MS, Nasr NT, Khalifa R, Mahdi AH, Mahmoud WS. 1978. Cats as reservoir hosts of *Toxocara* and other parasites potentially transmissible to man in Egypt. Acta Parasitol Pol (25): 383-389.

Arya LK, Rathinam SR, Lalitha P, Kim UR, Ghatani S, Tandon V. 2016. Trematode Fluke *Procerovum varium* as Cause of Ocular Inflammation in Children, South India. Emerging infectious diseases 22(2):192-200.

Ashford RW, Crewe W. 2003. The parasites of *Homo sapiens* An Annotated Checklist of the Protozoa, Helminths and Arthropods for Which We Are Home (2nd ed.), Taylor & Francis Group, London and New York.

Attia MM, Elgendy MY, Abdelsalam M, Azza Hassan A, Prince A, Salaeh NM, Younis NA. 2021. Morpho-molecular identification of *Heterophyes heterophyes* encysted metacercariae and its immunological and histopathological effects on farmed Mugil cephalus in Egypt. Aquaculture Int (29):1393-1407.

Chai JY. 2007. Intestinal flukes. In: Murrell K.D., Fried B., editors. Food-borne Parasitic Zoonoses, Fish and Plant-borne Parasites. Springer; New York, pp. 53–115.

Chai JY. 2014. Helminth-trematode. *Heterophyes heterophyes* Encyclopedia of Food Safety, 2, Elsevier 158-163.

Chai JY, Lee SH 2002. Food-borne intestinal trematode infections in the Republic of Korea. Parasitol Int. (51):129-154.

Chai JY, Seo BS, Lee SH, Hong ST. 1986. Growth and development of Pygidiopsis summa in rats and mice with a supplementary note on its morphological characters. Korean J Parasitol 24(1): 55-62.

Chai JY, Sohn WM, Na BK, Yong TS, Eom KS, Yoon CH, Hoang EH, Jeoung HG, Socheat D. 2014. Zoonotic trematode metacercariae in fish from Phnom Penh and Pursat, Cambodia. Korean J Parasitol (52): 35-40.

Dung DT, De NV, Waikagul J, Dalsgaard A, Chai JY, Sohn WM, Murrell D. 2007. Fish-borne zoonotic intestinal trematodes, Vietnam. Emerg Infect Dis. (13):1828-1833.

El-Azazy OM, Abdou NM, Khalil AL, Al-Batel MK, Majeed QA. 2015. Potential zoonotic trematodes recovered in stray cats from Kuwait municipality, Kuwait. Korean J Parasitol 53(3): 279-287.

El-Dakhly Kh M, El-Nahass E, Uni S, Tuji H, Sakai H. 2012. Levels of infection of gastric nematodes in a flock of great cormorants (Phalacrocorax carbo) from Lake Biwa, Japan. J Helminthol 86(1): 54-63

El-Dakhly Kh M, Aboshinaf ASM, El-Nahass E, Gharib AF. 2017. Preliminary study on the helminth fauna in necropsied stray cats (*Felis catus*) in Beni-Suef, Egypt. J Adv Vet Res 7(4): 87-92.

El-Dakhly Kh M, Aboshinaf ASM, Mahrous LN, Gharib AF. 2020. The Occurrence and Diversity of Intestinal Digenetic Trematodes in Stray Cats, Felis Catus, In Egypt. Int J Zoo Animal Biol, 3(2): 000214.

El-Seify MA, Aggour MG, Sultan K, Marey NM. 2017. Gastrointestinal helminthes of stray cats in Alexandria, Egypt: A fecal examination survey study. *Vet Parasitol Reg Stud Reports (*8):104–106.

El-Shahawi GAZ. 1983. Studies on some fishes as intermediate hosts of helminth parasites of some birds and mammals. Master Degree of Science, Fac. of Sci. El-Minia Univ.

Floyd M. 1991. The Modified Triadan System. J Vet Den 19-20.

Frihy OE, Dewidar KM, El Raey MM 1996. Evaluation of coastal problems at Alexandria, Egypt. *Ocean & Coastal Management* 30(2–3):281–95.

Henedi A. 2019. First molecular identification

of adult *Heterophyes heterophyes* and *Heterophyes dispar* (Digena: *Heterophyidae*) from Kuwaiti stray cats using ITS2 sequence. Int J Appl Nat Sci (8):9-20.

Khalil MB. 1933. The life history of the human trematode parasite *Heterophyes heterophyes* in Egypt. (222): 537-38.

Kruse OW, Pitchard MH. 1982. The collection and preservation of animal parasites. Univ. of Nebraska Press, Nebraska, U.S.A.

Lee SU, Huh S, Sohn WM, Chai JY. 2004. Sequence comparisons of 28S ribosomal DNA and mitochondrial cytochrome c oxidase sub unit I of Metagonimus yokogawai,M. takahashii and M. miyatai. Korean J Parasitol (42):129–135.

Mahanta J, Narain K, Srivastava VK. 1995. Heterophyid eggs in human stool samples in Assam: first report for India. J Commun Dis (27): 142-145.

Marey NM. 2016. Some studies on parasites of cats. MVs Thesis, Faculty of Veterinary Medicine Kafrelsheikh University, Egypt.

Masala S, Piras MC, Sanna D, Chai JY, Jung BK, Sohn WM, Garippa G, Merella P. 2016. Epidemiological and molecular data on heterophyid trematode metacercariae found in the muscle of grey mullets (Osteichthyes: Mugilidae) from Sardinia (western Mediterranean Sea). Parasitol Res 115(9):3409-3417.

Millan J, Casanova J. 2009. High prevalence of helminth parasites in feral cats in Majorca Island (Spain). Parasitol Res (106): 183-188.

Pearson JC. 1999. Proposed division of the genus *Heterophyes* Cobbold, 1865 (Trematoda: *Heterophyidae*). Syst Parasitol (43):11-15.

Pica R, Castellano C, Cilia C, Errico FP 2003. Intestinal fluke infections: the heterophyids. Clin Ter (154): 61-63.

Shin SS, Oh DS, Ahn KS, Cho SH, Lee WJ, Na BK, Sohn Wm 2015. Zoonotic Intestinal Trematodes in Stray Cats (*Felis catus*) from Riverside Areas of the Republic of Korea. Korean J Parasitol, 53(2):209-213.

Soulsby EJL. 1982. Helminthes, Arthropods and Protozoa of domesticated animals. 7th Edn., London, Bailliere, Tindall and Cassel.

Tamura K, Stecher G, Peterson D, Filipski A , Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol (30): 2725-2729.

Thabit HTM. 2011. Biological and parasitological studies on some endoparasites of cats in Assiut, Egypt. PhD. Thesis, Faculty of Veterinary Medicine Assiut University, Egypt.

Thaenkham U, Nawa Y, Blair D, Pakdee W. 2011. Confirmation of the paraphyletic relationship between families *Opisthorchiidae* and *Heterophyidae* using small and large subunit ribosomal DNA sequences. Parasitol Int (60):521–523.

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22(22):4673-4680.

Woo PTK. 2006. Fish diseases and Disorders.V.I. Protozoan and Metazoan infections. 2nd edition.UK. CABI Publishing.

Yamaguti S. 1958. Systema Helminthum, Vol. I. Digenetic Trematodes, Part I and II. Inter Science Publishers Inc. New York. 1575, pp 106 pl.

Yu SH, Mott KE. 1994. Epidemiology and morbidity of food-borne intestinal trematode infections. Trop Dis Bull 91 R125-R152.