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Prevalence of Monogenetic and Digenetic Trematodes Parasitized Fish Collected from Port Said and Ismailia Governorates, Egypt

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ABSTRACT

Trematodes are the most varied group of fish parasites. The present study investigated the trematode infection of fish collected from Port Said and IsmailiaGovernorate, Egypt during the period from March 2022 to February 2023. A total number of 360 fish of nine species were randomly collected. The parasitological examination showed that a total of 11 monogenetic trematodes were isolated from the gills, skin, and fins of different fish spp. (*Cemocotyle carangis*, *Pricea multae*, *Furnestinia echeneis*, *Tetrancistrum indicum*, *paranella diplodae*, *Microcotyle chrysophrii*, *Calceostoma Calceostom* and four different species of *Diplectanum*). In addition, four digenetic trematodes were isolated from the gastrointestinal tract of *Dicentrarchus labrax* (*Acanthostomum spiniceps*, *Gonocerca trematomi*, *Erilepturus hamate*, *Metadena crassulata)*,and*Transversotrema* spp. from the skin besides two encysted metacercaria from liver and muscle. Furthermore, *Opisthadena* spp. from*Sardinella* spp., *Hexangium sigani* from the gastrointestinal tract of *Siganus revulatus*, *Clinostomum complanatum* metacercaria from the branchial cavity of *Oreochromis niloticus* and *Tangiopsis chinensis* from the gastrointestinal tract of *Epinephelus aeneus* were isolated. The present study revealed high parasite diversity which has dangerous impacts on fish.

INTRODUCTION

Fish parasites can cause various issues, potentially leading to death. These problems include physiological harms such as cell proliferation, immunomodulation, negative behavioral reactions, altered growth patterns, and tissue replacement. Additionally, parasites can also adversely affect reproduction in fish **(Iwanowicz, 2011)**. The gills of fish are invaded by several parasite species; they can be seen between the gill filaments or on the gill arches. The infested fish may have severe erosion, extensive discolorations (typically paler), many white patches, and increased mucus secretion in more serious situations **(Toksen, 2007**). Proliferative cell alterations, such as significant epithelial hyperplasia (lamellar gill fusion), hypertrophy, edema, and inter-lamellar vesicle development are frequently brought on by parasite colonization of the gills **(Taylor *et al.*, 2009)**.

The most known infections affecting fish are parasitic infections with Trematode (monogenea and digenea). Monogenetic trematodes are flatworms or flukes that live as parasites on the surface of their fish hosts. They've been identified as a major fish disease in aquaculture **(Faruk, 2018)**. Monogeneans have a direct single host life cycle and can reproduce quickly in high density aquaculture situations since they don't need an intermediate host **(Rohde, 1993)**. Fish parasites can cause severe damage to the fish gills, obstructing the exchange of breathing gases and ions, which increases the risk of mortality **(Stephens *et al.*, 2003)**. In terms of host specificity, monogeneans are quite specialized. They can be hermaphrodites, viviparous or oviparous, and frequently protandrous. They attach to their host via a specialized muscular organ called the opisthaptor, which contains a variety of sclerotized hooks, connecting bars, clamps, and epidermal structures **(Paladini *et al*., 2017)**. Digenetic trematodes, popularly known as "flukes", are endo parasitic flatworms that primarily affect the alimentary canal or related organs. Miracidium, sporocyst, redia, cercaria, metacercaria, and adult are the main developmental stages of digeneans **(Faruk, 2018)**. Their indirect life cycle is complex and involves at least one intermediary host. The larval metacercaria, which can be found in tissue inside a cyst or unencysted depending on the species, is the stage that fish meet the most frequently. The first intermediate host, which is typically represented by a snail, is where the miracidium begins its life cycle. The miracidium is typically ovoid and has cilia covering its body, allowing it to swim and look for it. A piscivorous bird is often the last host. When cercarial larvae first enter the fish through its skin, damage to the fish may result **(Paladini *et al*., 2017)**. The aim of the present study was directed towards further understanding the prevalence of trematode infections of some collected fish from Port Said and Ismailia.

MATERIALS AND METHODS

1. Sample collection and clinical examination

A total of 360 fish from nine species were collected during the study period from March 2022 to February 2023. Among these, eight species were marine fish, totaling 320 individuals, including 40 *Pomadasys stridens*, 40 *Siganus revulatus*, 40 *Sparus aurata*, 40 *Scomberomorous cavalla*, 40 *Argyrosomus regius*, 40 *Epinephelus aeneus*, 40 *Dicentrarchus labrax*, and 40 *Sardinella* spp. Additionally, one freshwater fish species, *Oreochromis niloticus* (40 individuals), was collected. Ten fish from each species were examined per season. The specimens were captured alive from Lake Manzala and the Mediterranean coast in Port Said, as well as Lake Timsah in Ismailia Governorate, with the assistance of fishermen and appropriate fishing gear. The captured fish were transported alive to the Parasitology Laboratory, Faculty of Veterinary Medicine, Suez Canal University, and to the Central Lab for Marine Fish Diagnosis and Measurement of Fish and Water Quality (MADE2) at Ashtoum Elgameel. They were transported in polyethylene bags, containing ⅓ of their volume in seawater, with the remaining volume filled with air.

2. Clinical examination

Before dissection, the body weight and total length of the investigated fish species were measured using an electronic balance. This was followed by a clinical assessment of

live fish. According to **Amlacker (1970)**, visual examination of the fish samples under investigation was done to check for any clinical abnormalities.

3. Parasitological examination

Fish samples were macroscopically examined immediately after sacrificing, following the method described by **John (1966)**. This examination involved the identification of any abnormalities in different sections of the fish body using naked eyes and hand magnifiers. Additionally, the samples were microscopically examined to detect both internal and external parasites.

3.1. Examination of skin and fins

Fish skin and fins sacrificed were inspected for external parasite using both the naked eye and a magnifying glass. They were put on a dissecting dish and scraped with a scalpel from just beyond the operculum to the tip of the tail. Fins and scales were then transferred to slides with a drop of distilled water and a cover slip to prevent drying, and they were examined under a microscope.

3.2. Examination of gills

The opercula were carefully cut away using scissors, exposing the gills. Subsequently, the gills on both sides were removed once by making cuts at their extremities. The gills were then examined with the naked eye and a magnifying glass to check for any parasitic infestation.

The gill lamellae were separated by slicing the cartilaginous arch away with needles after extracting the gill arche and were put on a microscope slide. A few water droplets were sprinkled on the slide to verify that the filaments were evenly distributed under the whole cover slip **(Lucky, 1977)**. Gill mucus was also transferred to slides and checked for parasites using a drop of distilled water and a microscope.

3.3. Examination of the mouth cavity

With the use of a hand lens and the naked eye, the oral cavity was examined for the existence of any anomalies or adherent parasites.

3.4. Examination of gastrointestinal tract

In a Petri dish filled with physiological saline, the stomach and intestine were separated individually, and the gastrointestinal tract and gall bladder were visually inspected for the presence of encysted metacercaria or worms. The edge of the slide was used to squeeze the content of GIT before being thoroughly washed with physiological saline solution. Then, the worm was selected and transferred into a test tube filled with physiological saline. The tube was forcefully shaken to remove the parasites' slime and debris. Afterwards, it was transferred into suitable preservative and kept in refrigerator for complete relaxation of the worm. Finally, samples were examined using a dissecting binocular microscope.

3.5. Examination of musculature, kidney, liver, spleen and heart

In order to check for encysted metacercaria or worms, little pieces of each internal organ and the musculature were removed, mixed with a few drops of saline, and pressed between two glass slides.

4. Permanent slides smear preparations and staining

Monogenetic trematode

The collected worms were cleaned with physiological saline on many occasions to remove dirt and mucus before being placed in the refrigerator to completely rest.

Between the cover and the glass slide, they were then gently crushed. The worms were fixed in 10% buffered neutral formalin for 12–24 hours, washed several times in distilled water to remove excess fixative, stained in alum carmine for an overnight period, washed in tap water, dis-stained in 1% acid alcohol, and dehydrated in ascending series of ethyl alcohol (30%, 50%, 70%, 80%, 90%, 100%). Finally, the specimens were cleaned with clove oil, cleaned with xylene to remove the oil, mounted in Canada balsam, and allowed to dry in a horizontal position **(Pritchard & Kruse, 1982)**.

Digenetic trematodes

Water- filled test tubes with corked lids were filled with endoparasites from the stomach and intestines, and they were forcefully shaken (the worm became washed and fatigued and did not contract again during fixation). The trematodes were gently squeezed between two glass slides or, depending on their size between a glass slide and a cover slip under the microscope until the parasite's clearance level and internal details were clearly visible. Following this, they were fixed using 5-10% formalin solution, adjusted based on the thickness of the digenea, and left to fix for 24 hours. The slides were carefully separated from each other by gently putting them in a petri dish filled with water to prevent damage. Subsequently, the specimens were dyed with Semichon's acetocarmine for five minutes. After staining, the specimens were gently washed in distilled water, dehydrated in an ascending series of ethyl alcohol concentrations (30%, 50%, 70%, 80%, 90%, 100%), cleared with clove oil, mounted in Canada balsam, and then allowed to dry in a horizontal position **(Negm Eldin & Saleh, 1995)**.

5. Identification of the isolated parasites

Monogenetic trematodes were identified according to **Yamaguti (1934)**, **Yamaguti (1958)** and **Pamplona-Basilio *et al.* (2011)**. Moreover, the digenetic trematodes were identified according to **Yamaguti (1934)** and **Nahhas *et al*. (1998)**.

6. Statistical analysis

The analysis was carried out with the help of IBM SPSS® Statistics version 26 (IBM® Corp., Armonk, NY, USA). In qualitative data, frequency and percentage were used as units of measurement. To look at the relationship between qualitative variables, Pearson's Chi-square test was utilized. All tests had two tails. The significance level was set at *P*-value< 0.05. Prevalence of trematodes were estimated using the following formula (number of individuals of a host species infected with trematode species ÷ number of hosts examined × 100).

Ethics

This study was approved by the Ethics Committee of the Suez Canal University. All animal experiments were conducted following the guidelines of the Guide for the Care and Use of Laboratory Animals, Faculty of Veterinary Medicine Science, Suez Canal University, Egypt (Approval No. 2020056).

RESULTS

Total prevalence of trematode infections among the examined fish all over the year

The overall prevalence of trematode infections across the four seasons was 48.1%. The highest infection rate was observed in *Siganus revulatus* (90%), followed by *Pomadasys stridens* (87.5%), *Dicentrarchus labrax* (72.5%), *Scomberomorous cavalla* (52.5%), and *Sparus aurata* and *Argyrosomus regius*, both with the same percentage (37.5%). *Sardinella* spp. exhibited an infection rate of 27.5%, while *Epinephelus* aeneus showed a rate of 17.5%. The lowest infection rate was found in *Oreochromis niloticus* (10%) (Fig. 1).

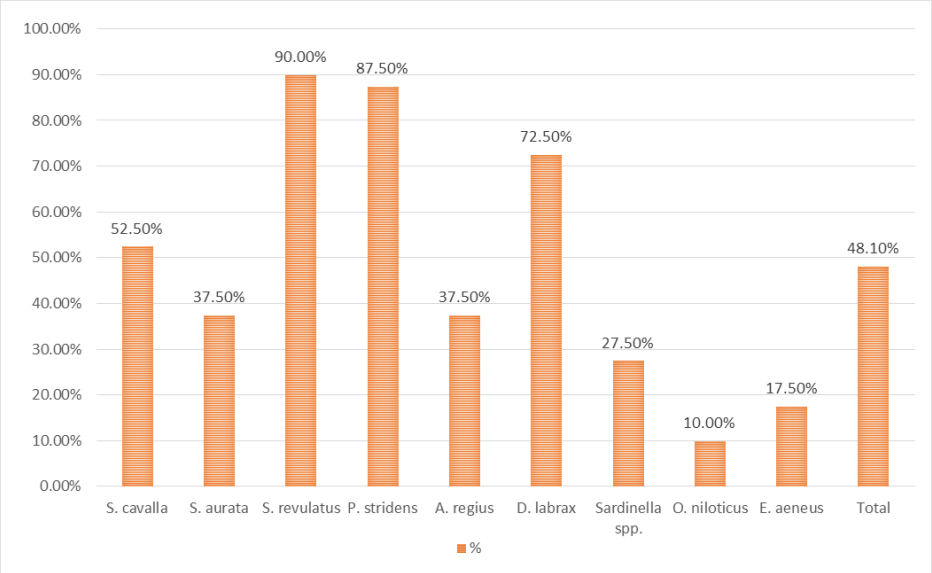

Fig. 1. Prevalence of trematode infection among the examined fish all over the year

Total prevalence of monogenetic and digenetic trematodes among the examined fish

Tables (1, 2) reveal that, the total prevalence of monogenean infestations (*Pricea multae*, *Cemocotyle carangis*, *Furnestinia echeneis*, *Furnestinia echeneis*, *Tetrancistrum indicum, paranella diplodae*, *Microcotyle chrysophrii*, *Diplectanum* spp., *Diplectanum aequans*, *Diplectanum squamatum*, *Diplectanum copiosum* and *Calceostoma Calceostoma*) was the highest (59.5%) (Figs. 2, 3). In addition, the total prevalence of digenean infections (*Acanthostomum spiniceps*, *Gonocerca trematomi*, *Erilepturus hamate*, *Metadena crassulata*, encysted metacercaria in liver, encysted metacercaria in muscle, *Transversotrema* spp., *Clinostomum complanatum metacercaria*, *Tangiopsis chinensis*, *Opisthadena bodegensis*, *Opisthadena dimidia* and *Hexangium sigani*) was the lowest (27%) (Figs. 4, 5).

Table 1. Total prevalence of monogenetic trematodes among the examined fish

Fish species	Parasite	Stage	Site	N.	prevalence
S. cavalla	*Pricea multae*	Adult	Gills	119	59.5%
	Cemocotyle carangis	Adult	Gills		
S. aurata	*Furnestinia echeneis*	Adult	Gills		
S. revulatus	*Tetrancistrum indicum*	Adult	Gills		
	paranella diplodae	Adult	Gills		
P. stridens	*Microcotyle chrysophrii*	Adult	Gills		
A. regius	*Diplectanum spp.*	Adult	Gills		
	Diplectanum aequans	Adult	Gills		
	Diplectanum squamatum	Adult	Gills		
	Diplectanum copiosum	Adult	Gills		
	Calceostoma calceostoma	Adult	Skin, fins		

Table 2. Total prevalence of digenetic trematodes among the examined fish

Fish species	Parasite	Stage	Site	N.	prevalence
D. labrax	*Acanthostomum spiniceps*	Adult	GIT	54	27%
	Gonocerca trematomi	Adult	GIT		
	Erilepturus hamati	Adult	GIT		
	Metadena crassulata	Adult	GIT		
	Encysted metacercaria	Larvae	Liver		
	Encysted metacercaria	Larvae	Muscle		
	Transversotrema spp.	Adult	Skin		
Sardinella spp.	*Opisthadena bodegensis*	Adult	GIT		
	Opisthadena dimidia	Adult	GIT		
S. revulatus	*Hexangium sigani*	Adult	GIT		
O. niloticus	*Clinostomum complanatum*	Larvae	Branchial cavity		
E. aeneus	*Tangiopsis chinensis*	Adult	GIT		

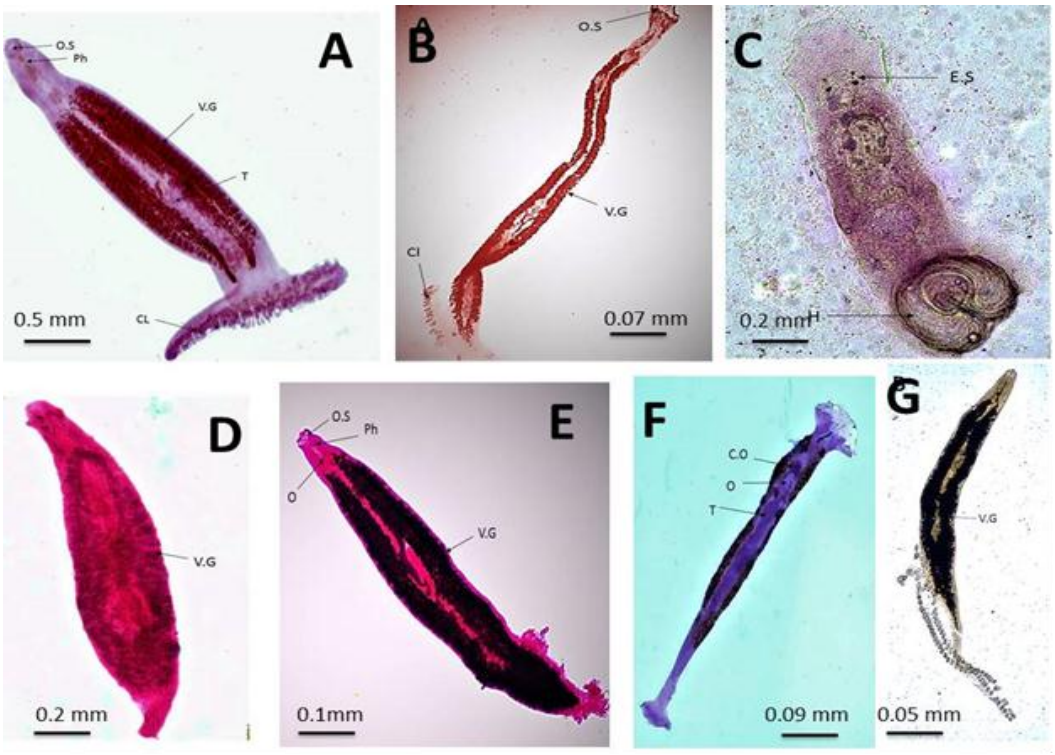

Fig. 2. Photomicrographs of the adult monogenea parasites showing: (A) *Pricea multae;* (B) *Cemocotyle carangis; (*C) *Furnestinia echeneis; (*D) *Tetrancistrum indicum;* (E) *Paranella diplodae; (*F) *Calceostoma calceostoma; (*G) *Microcotyle chrysophrii;* (O.S) Oral Sucker; (Ph) pharynx; (O) Oesophagus; (V.G) Vitelline gland; (Cl) Clamps; (T) Testis; (E.S) Eye spot, and (C.O) Copulatory organs

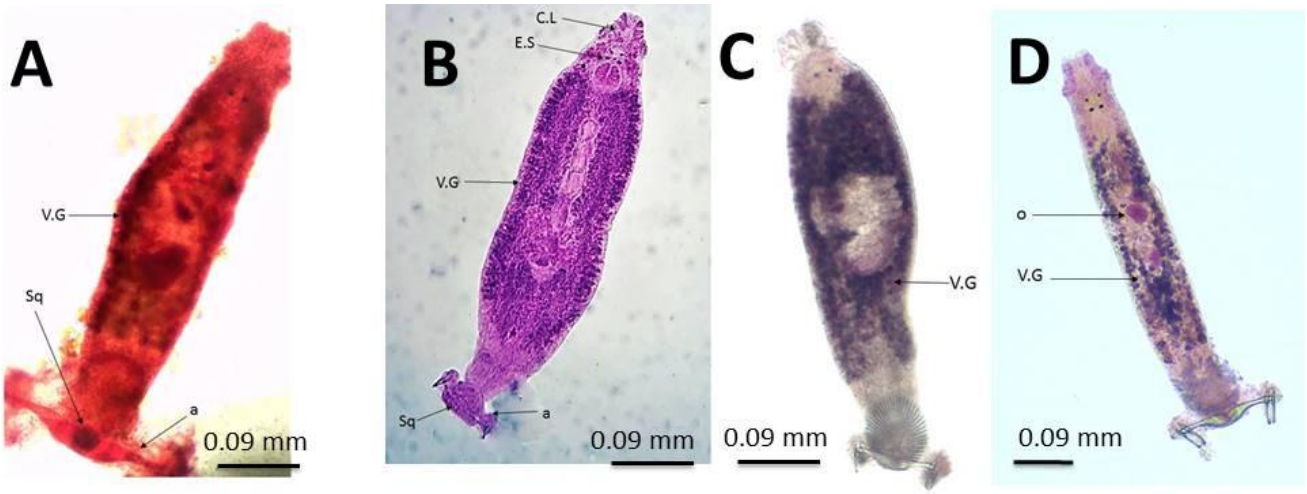

Fig. 3. Photomicrographs of the adult monognean parasites showing: (A) *Diplectanum* spp.; (B) *Diplectanum aequans*; (C) *Diplectanum squamatum*, and (D) *Diplectanum copiosum*

(a) Anchor; (Sq) squamodisc; (V.G) Vitelline gland; (C.L) Cephalic lobe; (E.S) Eye spot; (O) Ovary

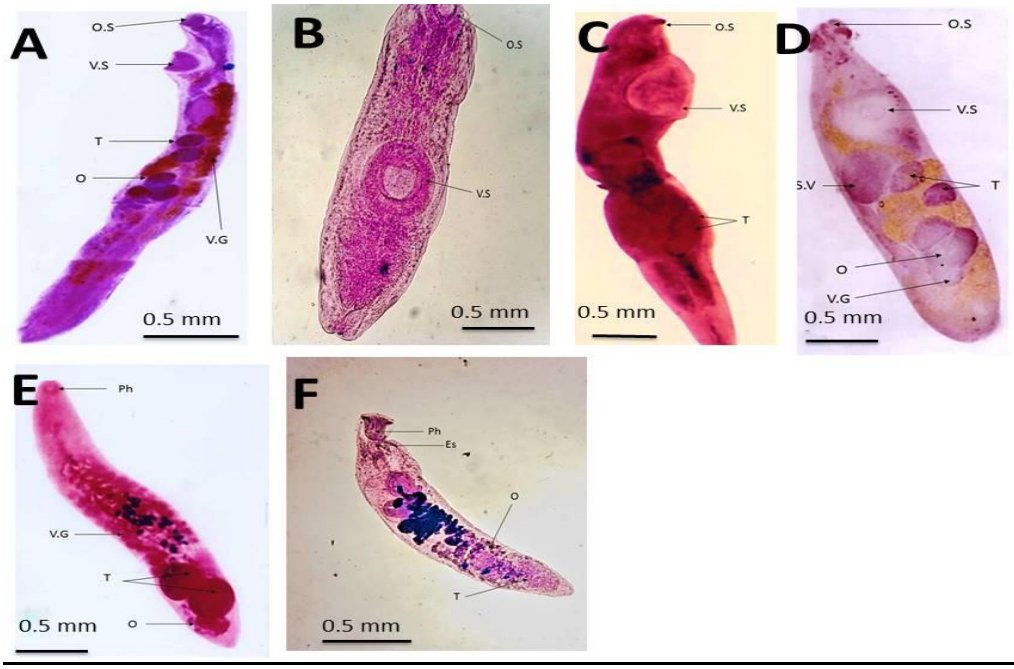

Fig. 4. Photomicrographs of the adult digenean parasites showing: (A) *Opisthadena dimidia*; (B) *Gonocerca trematomi*; (C) *Erilepturus hamate*; (D) *Opisthadena bodegensis*; (E) *Hexangium sigani*; (F) *Acanthostomum spinicep*
(O.S) Oral sucker; (V.S) Ventral sucker; (O) Ovary; (V.G) Vitelline gland; (T) Testis; (S.V) Seminal vesicle; (Ph) Pharynx; (Es) Esophagus .

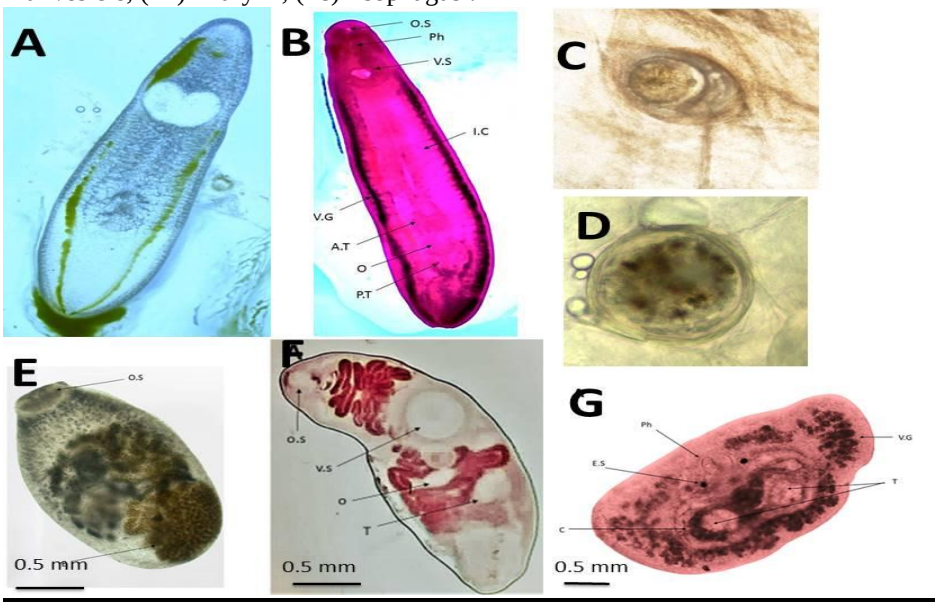

Fig. 5. Photomicrographs of the digenean parasites showing: (A & B) *Clinostomum complanatum* metacercaria; (C) Encysted metacercaria in liver; (D) Encysted metacercaria in muscle; (E) *Metadena crassulata*; (F) *Tangiopsis chinensis*, and (G) *Transversotrema* spp.(O.S) Oral Sucker; (Ph) Pharynx; (V.S) Ventral sucker; (I.C) Intestinal caeca; (V.G) Vitelline gland; (A.T) Anterior testis; (O) Ovary; (P.T) Posterior testis; (T) Testis; (Ph) Pharynx; (C) Caecum; (E.S) Eye spot

DISCUSSION

Trematodes represent the most diverse group of fish parasites, being present in either larval or adult forms in the majority of fish species **(Rajput & Langer, 2022)**. The objective of this study was to determine the prevalent trematode species parasitizing various fish species, including *Scomberomorus cavalla*, *Sparus aurata*, *Siganus revulatus*, *Pomadasys stridens*, *Argyrosomus regius*, *Dicentrarchus labrax*, *Sardinella* spp., *Oreochromis niloticus*, and *Epinephelus aeneus* found in the regions of Port Said and Ismailia. In the present study, the total prevalence of trematode infection is 49.69%. This result is lower than what was obtained by **Hamouda *et al*. (2018)**, who reported a prevalence of 95%. On the other hand, this prevalence was higher than that reported by **Saleh (2010)**, **Dan-kishiya *et al*. (2013)**, **Eissa *et al*. (2013b)**, **El-Seify *et al*. (2017)**, and **Villar-Beltrán *et al*. (2020)**, who concluded overall prevalence rates of trematode infections as 24.2%, 2.9%, 32.2%, 27%, and 4.8%, respectively. These differences in the infection rates between studies may be due to ecological differences in the sites from which the fish were collected, as environmental conditions have a significant effect on parasitic infection rates **(Britton *et al*., 2011)**, and due to the differences in fish species **(Eissa *et al*., 2013a)** and **(Vasemägi *et al*., 2017)**.

The total prevalence of monogenean infestation was (59.5%), this result was nearly similar to **Yoon *et al*. (2015)** which was (63.8%) and **Eissa *et al*. (2017)** who reported that the prevalence was 55%. On the other hand, the current finding is lower than that obtained by **Morsy *et al*. (2011)** and **Ferreira-Sobrinho and Tavares-Dias (2016)** with values of 74.1% and 84.3%, respectively. While, it is higher than that obtained by **Fioravanti *et al*. (2006)**, **Quaglio *et al*. (2007)**, **Adaic (2012)**, **Bayoumy *et al*. (2012)**, **Mahmoud *et al*. (2014)**, **Shini *et al*. (2015)**, **Adawy *et al*. (2016)**, **Eissa *et al*. (2016)**, **Bendryman *et al*. (2017)**, **Eissa *et al*. (2020b)**, **Fakhry *et al*. (2020)**, **Neves *et al*. (2020)**, and **Workeale (2021)**, who recorded values (49.80%, 32.21%, 4.44%, 38.8%, 32%, 45%, 16.31%, 7.81%, 32%, 13%, 32.35%, 22.4% and 5.7%), respectively. This difference may be attributed to the species of isolated parasites, fish species, and the number of examined samples **(Mahmoud *et al*., 2011)**. Furthermore, it might reflect the difference in the habitat, behavior of fish, environmental conditions and also the type of diet and feeding habits (**Mwita & Nkwengulila, 2008; Nunn *et al*., 2008)**.

Concerning the total prevalence of digenetic trematode, it was 27%. This result is nearly similar to the results obtained by **Youssef and Derwa (2005)**, **Abdel-Mawla and El-Ekiaby (2012)** and **Abdel-Mawla and Yousef (2019)**, as the total prevalence of digenetic trematodes was (28.4%, 26% and 32.14%), respectively. Whereas, it is lower than that obtained by **El-Ashram and Shager (2008)** and **Abd-ELrahman *et al*. (2023)**, for it was recorded as 61.3% and 58%, respectively. On the other hand, the present value is higher than that obtained by **Abdel-Mawla and Abo-Esa (2011)**, **Abdel-Gaber *et al*. (2018)** and **Eissa *et al*. (2020a)**, which was (4.6%, 10.45% and 17.5%), respectively recorded. This variation in prevalence may not only be attributed to the change in the

climatic conditions but also to the differences in species of examined fish, size, feeding habits, species of digenetic parasites and the locality from which fish samples were collected **(Ekanem *et al.*, 2011; El-Shahawy *et al.*, 2017)**. In addition, this might be attributed to the type of intermediate host present and the prevailing physicochemical factors **(Paperna, 1980)**.

Regarding the parasitological examinations, 11 monogenetic trematodes were isolated from the gills, *Cemocotyle carangis, Pricea multae, Furnestinia echeneis, Tetrancistrum indicum, paranella diplodae, Microcotyle chrysophrii, Calceostoma Calceostoma,* and four different species of *Diplectanum.* In addition to the uforementioned species, four digenetic trematodes were isolated from gastrointestinal tract of *Dicentrarchus labrax* (*Acanthostomum spiniceps, Gonocerca trematomi, Erilepturus hamate, Metadena crassulata)*. The morphological description is in agreement with **Byrd (1963)**, **Fernandes *et al*. (2009**), **Al-Salim (2013), Abou Zaid *et al*. (2018)**, **Eissa *et al*. (2020a)** and **Aly *et al*. (2020)**.

Examination of *Dicentrarchus labrax* revealed the presence of two encysted metacercaria in the liver and muscle. **Hegazi *et al*. (2014)** also stated encysted metacercariae of Family Heterophyidae in fresh and brackish water fish in an endemic focus in Egypt. **Sayed *et al*. (2014)** detected *Cynodiplostomum* in muscular tissue only, while they found *Prohemistomum* in muscular tissue, liver, kidney and gills of examined *Claris gariepinus* fish. **Attia *et al*. (2021)** identified heterophyid encysted metacercariae infecting gray mullet *Mugil cephalus.*

Examination of *Dicentrarchus labrax* skin revealed ectoparasitic digenea *Transversotrema* spp. which was previously stated in the studies of **Witenberg (1944)** and **Fadel (2021)** who described *Transversotrema haasi*. **Hunter and Cribb (2012)** detected Transversotrematids similar to *T. licinum*, collected from a single *Chaetodon flavirostris* from New Caledonia. While, **Womble *et al*. (2015)** detected *Transversotrema patialense* on the skin (epidermal spaces beneath scales near pectoral fins) of zebrafish and **Abou Zaid *et al*. (2018)** detected *T. patialense* from the skin of *Dicentrarchus labrax* in the Mediterranean area.

Examination of gastrointestinal tract of *Siganus revulatus* revealed *Hexangium sigani*, which was described by **Goto and Ozaki (1929)**. **Hassanine *et al*. (2016)** also stated *Hexangium sigani* from the intestine of *Siganus rivulatus* from the Egyptian coast of the Gulf of Aqaba. Examination of gastrointestinal tract of *Sardinella* spp. revealed two species of *Opisthadena*. *Opisthadena bodegensis* and *Opisthadena dimidia,* which was differentiated by **Johnson and Copsey (1953)**. Furthermore, the morphological description agrees with that reported in the study of **Youssef and Derwa (2005)**.

The examination revealed *Clinostomum complanatum* metacercaria, which was isolated from the branchial cavity of *Oreochromis niloticus*. The results of the morphological features of the obtained metacercariae agree with the results of *C. complanatum* as described by **Rudolphi (1819)** and **Taher (2009)** from the same fish

species, **Caffara *et al*. (2011)** and **Wang *et al*. (2016)** from operculum, mandible, muscle, and oral cavity of cultivated ayu (*Plecoglossus altivelis*) and loach **(***Misgurnus anguillicaudatus***)** and also similar to **Salem *et al*. (2021)** who isolated it from the same fish species. *Tangiopsis chinensis* was isolated from gastro intestinal tract of *Epinephelus aeneus*; its morphological description is similar to that given by **Tang (1951)** and **Skrjabin (1955)**. It is supported by **Qorany (2020)**, who isolated it from the stomach and intestine of *D. labrax*.

CONCLUSION

Fish parasites can cause severe economic losses, the present study revealed that several monogenetic and digenetic trematodes were isolated from different fish species and the total prevalence of monogenean was higher than digenean. This high trematodes diversity has dangerous impacts on fish. Consequently, solutions should be found to decrease trematode infections.

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