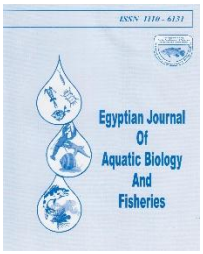
Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 24(5): 449 – 466 (2020)
www.ejabf.journals.ekb.eg



Survey on Some Ectoparasitosis in *Moolgarda seheli* from Suez Canal area, Egypt

Ismail A. Eissa[1], Mona M. Ismail [1], Mona Abdelwahab[2], Heba I. Abdel-Mawla[2], Amina A. Dessouki[3], Shimaa A. Fakhry[2] and Hassnaa Elsheshtawy[1]*

1. Dept. of Fish Diseases and Management, Fac. of Vet. Medicine, Suez Canal University.
2. Animal Health Research Institute, Ismailia Provrincal Branch-Agriculture Research Center.
3. Dept. of Pathology, Fac. of Vet. Medicine, Suez Canal Univ.

***Corresponding Author: hassnaa_elshshtawy@vet.suez.edu.eg**

ARTICLE INFO

Article History:
Received: July 3, 2020
Accepted: Aug. 14, 2020
Online: Oct. 10, 2020

Keywords:
Moolgarda seheli,
Suez Canal
Lernanthropus,
Benedenia,
phylogeny

ABSTRACT

This survey study has been applied to a total number of 200 *Moolgarda seheli* that collected randomly and seasonally from different areas of the Suez Canal at Ismailia Governorate. Infected fish showed no obvious clinical abnormalities except in case of heavy infestation with the ectoparasites, where some infested fishes revealed respiratory manifestation and surface swimming. Other fishes showed hemorrhagic areas on the gill cover, abdomen, and fins. The results of this survey revealed 2 phyla of economically important fish parasites which were three copepods (*Lernanthropus* species, *Caligus lichiae*, and *Lepeophtheirus lichiae*) isolated from the gills and one monogenean (*Benedenia sekii*) isolated from the gill and the buccal cavity of all examined fish. The total prevalence of infestation was 31% where *Lernanthropus*, *Caligus* species and monogenea were 24.5%, 5% and13%; respectively while the mixed infestation between crustaceans with monogenea was 7%. The seasonal prevalence of ectoparasitic infestation was the highest in winter (67.5%) followed by spring then autumn and the lowest was summer (10%). The highest infestation ofLernanthropus species was recorded in the winter (50%) and the lowest in summer (10%). On other hand, the highest infestation of *Caligus* was highest in the spring (33.33%) and disappeared in the other seasons. The seasonal prevalence of monogenean infestation was the highest in the spring (43.33%) and disappeared in the autumn and summer. Molecular identification of *Benedenia* species using PCR analysis of the 28SrRNA(mix of C1/D2 pairs) generated larger sequence fragments which were amplicons at approx. 800 bp. The partial sequencing and phylogenetics of the positive sample revealed that our sample was closer related to *Benedenia sekii* followed by *Benedenia sciaenae* from Australia which were 97.9% and 97.2%; respectively. The histopathological changes of infested fish with ectoparasite revealed severe atrophy of the gill lamellae along with mechanical destruction, other fish revealed focal infiltrations with leukocytes, in addition to congestion of the gill arch.

INTRODUCTION

The objectives of this research are to provide adequate knowledge about the most ectoparasitic diseases affecting *Moolgarda seheli* from Suez Canal area to detect

the clinical picture and post mortem lesions of these diseases on the infested fish. To study the total and seasonal prevalence's of these diseases. To benefit with PCR analysis by using 28 SrRNA (mix of C1/D2 pairs) as marker for identification of one fish parasite (*Benedenia* species) and phylogenesis with others in the same genera. To study histopathological alterations in infested fish with parasites.

Over the past few years in Egypt, marine sector of the aquaculture industry has been developed to cover the limitation of the freshwater resources usage. *Moolgarda seheli* belongs to family *Mugilidae* that consists around the world of more than 72 species from 17 fish genera. It is one of the commercially important fish species in Suez Bay and Egypt, although it has a lower growth rate, fetches a higher market price compared to the other mullet in Egypt because of its highly appreciated taste **(Nelson, 2006**). With the development of semi- intensive and intensive fish farming. The importance of parasites as disease-causing agents has become more evident, because of their ability to evolve rapidly the most serious limiting factors in aquaculture (**Antonelli *et al.*, 2016)**. Ectoparasite infestation lead to great economic losses due to not only resulted from direct harm to fish, but also from disfigurement which rendered fish grown for food and ornamental fish unsuitable for sale, thus impose a big loss to fish industry **(Piasecki *et al.*, 2004).**Crustacean diseases cause massive economic losses to fisheries not only from killing, stunting, or damaging these fishes, but also cause mortalities or impair immature fishes or are unfit for sale **(Ali *et al.*, 2015).** Copepods comprise the largest group of crustacean parasites on fish causing economical loss **(Jithendran *et al.*, 2008)** such as caligus species as mentioned by **Boxshall and El-Rashidy (2009).** Also, the presence of more than 100 species of the gill parasite Lernanthropus can cause high mortality in small juvenile of the European sea bass (*Dicentrarchus labrax*).**(Athanassopoulou *et al.*, 2001**)and in *Mugil cephalus* and *Moolgarda seheli* **(Hassanin, 2016).** Monogeneans have been considered as a factor limiting aquaculture productivity as it frequently causes mixed infections with other parasites and secondary bacterial infections **(Antonelli *et al.*, 2010)** such as *Neobenedenia* which considered as harmful ectoparasite that can cause disease and mortality in aquaculture systems **(Brazenor *et al.*, 2018).** The external parasites have various patterns in spatial distribution, patterns that influence climate change. Climate change may be conducive to the production and distribution of parasites. This could have economic impacts on marine ecosystem function and structure **(Morris & Costello, 2019)**.mostly monogenetic infestation in marine aquaculture systems increased in summer season and decreased in winter season **(Bayoumy, 2003; Abdel-Mawla & Shalaby, 2014)** ,others proved that spring season is the highest season as **El-Lamie (2007) and Eissa *et al.* (2016, a)** . The crustaceans attachment sites were the body surface and gill cavities of the fish **(Maran *et al.*, 2009)** with higher infestation in spring season and lower in winter one **(Faisal, 2008).**Clinical sigs appeared in infested fishes associated with intensity of parasites as low intensity showed no clinical sigs, no harm on fish but when fish are crowded or stressed, and water quality deteriorates,

parasites multiply rapidly and cause serious damage. Typically, heavily infected fish don't eat well and exhibit low growth rate, discoloration and mucus secretion. Weakened fish become more susceptible to opportunistic bacterial pathogens presented in the water and became pathogenic microorganisms that resulting in major stock losses **(Kayis *et al.*, 2009).** The ability of scientists to describe and define biodiversity in parasitology has been transformed by molecular techniques (primarily DNA sequencing) (**Bickford *et al.*, 2007; Detwiler *et al.*, 2010; Padial *et al.*, 2010; Sepúlveda & González, 2019)**. DNA barcoding, one of the best of genetic measures, provides a means of identifying known species by comparing sequence similarities, and has shown strong resolution at the species level for different parasite groups such as monogenea **(Chaabane *et al.*, 2016 ; Sepúlveda & González, 2019).**

2. MATERIALS AND METHODS

2.1.Fish:

A total number of 200 of *Moolgarda seheli* of different lengths and various body weights were collected randomly and seasonally from different areas of Suez Canal at Ismailia Province. The collected fish samples were identified according to Langdon and Jones (2002)**.**

2.2.Clinical picture:

Clinical examination has been done on the live or freshly dead fish. Fish specimens were grossly examined for detection of any ectoparasites and any clinical abnormalities according to **Amlacker (1970)** and the postmortem examination according to the methods described by **Lucky (1977)**

2.3.Parasitological examination:

Fish specimens were examined macroscopically and microscopically then collected crustacean parasites were stained and fixed according to **Lucky (1977)** while monogenetic trematodes were stained and fixed according to **Pritchard and Kruse (1982)** .

2.4.Molecular detection of *Benedenia* species (Monogenetic trematodes):

The genomic DNA extraction and purification of the parasite was performed using the procedures provided by ABT DNA Mini Extraction Kit (spin Column) (Catalogue number: ABT001) of the collected parasites preserved in 70% ethanol in Eppendorf tube Extracted DNA was stored in hydration solution at 4 ◦C. PCR amplification for the ribosomal 28S RNA 28S partial sequences from purified genomic DNA using specific primer combinations C1/D2 (approx.800 bp) where C1 (5′-ACCCGCTGAATTTAAGCAT- 3′) as forward primer and D2 (5′-TGGTCCGTGTTTCAAGAC - 3′) as reverse primer, as discussed by **Chisholm *et al.* (2001); Perkins *et al.* (2009); Moreira *et al.* (2019) .** PCR was carried out using of 50 μL of total reaction volume (2X Red master mix1X: Forward primer 20 picomole, Reverse primer20 picomole, DNA extract100 ng, Nuclease free water Up to 50µL). Standard cycle conditions for PCR were set as initial denaturation for 3 min at 95◦C, followed by 35 cycles of 30 s at 95◦C, 30 s at 50◦C, 90 s at 72◦C and final extension of 5 minutes at 72◦C. To confirm the targeted PCR amplification, five µl of the PCR product was electrophoresed along with 100bp DNA molecular weight 1% agarose gel containing

ethidium bromide (at the rate of 0.5µg/ml) at constant 80V for 30 min in 1X TAE buffer. The amplified product was visualized as a single compact band of expected size under UV light and documented by Samsung Note 4 smart phone

Sequencing of the PCR products using the amplified PCR products were submitted to Solgent Co Ltd (South korea) for gel purification and sequencing. PCR using Solgent EF-Taq, PCR Machine name: 9700(ABI).

2.5.DNA Alignment and Phylogenetic Analysis:

Nucleotide sequences were downloaded from GenBank and aligned with the identified sequences, using MAFFT alignment. Phylogenetic trees were constructed using the Neighbor joining method**,** employing the Kimura 2-parameter method. The trees were assessed using 1000 bootstrap replicates. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The optimal tree with the sum of branch length = 1.01 is shown. This analysis involved 48 nucleotide sequences. All ambiguous positions were removed for each sequence pair. Subsequently, the 28S rRNA alignment was trimmed to remove sequences that could not be aligned unambiguously and sequences from the genes concatenated to give a final alignment length 397 positions in the final dataset. Evolutionary analyses were conducted in MEGA **.(Kumar *et al.*, 2018)**

2.6.Histopathological examination- :

Specimens for histopathological techniques were freshly taken from affected gills of naturally infested fish. Specimens were trimmed and fixed in 10% phosphate buffered formalin, processed routinely and stained with H&E stain then examined **(Roberts, 2001).**

RESULTS

3.1.Clinical picture and postmortem finding:

The clinical examination of naturally infected *Moolgarda seheli* showed no noticeable clinical abnormalities except in case of heavy infestation with the copepod crustacean parasites. Some infested fish revealed respiratory manifestation, surface swimming and opened mouth. Other fish showed hemorrhagic area on gill cover, abdomen and fins. In some cases, the *Lernanthropus* species was seen by the naked eye (egg sac of copepods) in the form of black lines among the gill filaments (Figure 1.D). In case of family Capsalidae large number of visible, white colored parasites were found attached to the gill filaments and/or buccal cavity by its haptors. Infected gills were covered with mucous layer. Heavily infested gill showed excessive mucos secretions and paleness (Figure 1. A, B&C).

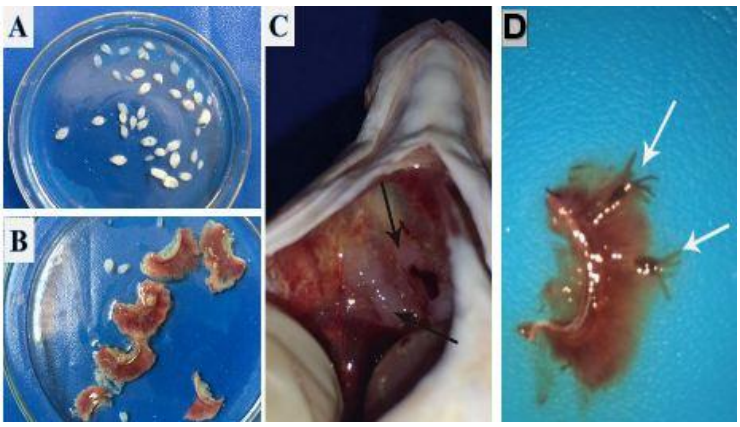


Figure 1:showing ectoparasitic infestation in the *Moolgarda seheli* as **A,B**: *Benedenia* species. in petri dish collected from different *M.seheli* **C**: buccal cavity and gills operculum showing Benedenia sp. (arrows). **D**: gill showing egg sacs of females *Lernanthropus* species.

Parasitological examination of all examined fish revealed 3 copepods parasites 1 species of family: Lernanthropidae (*Lernanthropus* species) isolated from the gills of *Moolgarda seheli* was the same as described by **Blainville (1822)** .Mandible was slender had 7 denticles. The first maxilla consists of 3 segments; the terminal is conical, and the basal segment has 2 distal broad spines. The second maxilla consists of segments; the terminal segment was provided with 2 rows of blunt teeth and blunt spines on the inner margins. The third segment had single distal spine. The exopod had 5 short distal spines, while the endopod had slender bristled seta. The 5^{th} legs were absent. The female is somewhat cylindrical and measured 3.1 mm and 1 mm in width at the middle of the body. The head separated by a constriction from the rest of the body. The egg strings are elongated and uniseriate, strongly flattened eggs (Figure 2., a &b). Other 2 species of family: Caligidae (one of genus caligus: *Caligus lichiae* and other one genus Lepeophtheirus*: Lepeophtheirus lichiae*). *Caligus lichiae*_is a crustacean parasite was isolated from the gills of infested fishes. These species can be distinguished by posterolateral corners of genital complex projecting laterally; female maxilliped with small myxal process. the corpus of the male maxilliped has three triangular processes along the myxal margin. The fourth process is minute and located proximally on the myxal margin the raised array of denticles on the ventral surface of the apron of leg 3 comprised 14–19 denticles (Figure 3. F). It was the same described by **Brian (1906)** . *Lepeophtheirus lichiae* was isolated from the gills of the infested fishes .Their morphological characters were as described by **Barnard (1948)** .Adult female characterized by caligiform body, comprising cephalothorax incorporating first to third pedigerous somites, free fourth pedigerous somite, genital complex and 1-segmented abdomen. Dorsal cephalothoracic shield subcircular, excluding marginal membranes, lateral margins evenly convex and ornamented with array of about 14 smalls dorsal sensilla plus 13 small compound sensilla beneath marginal membrane along each side, anteromedial part of frontal plate ornamented with numerous sensilla. The most obvious distinguishing character of *L. lichiae* is the extreme development of the spiniform fifth leg where it was conical, spinous process located behind lobate posterolateral corner of genital complex and extending posteriorly slightly beyond mid-length of caudal ramus. The length of spinous fifth leg was about 83% of length of genital complex **(Sakarya *et al.*, 2019)** (Figure 3., G&H). Also one monogenetic trematodes of family: Capsalidae (*Benedenia sekii*) was isolated from the gill and oral cavity of *M. seheli* with range (2-7) per fish. *Benedenia sekii* was characterized by broad body where body margin extending to anterior edge of anterior attachment organs. Pharynx was larger than anteriora ttachment organs. Haptor was larger, circular and equipped with relatively small accessory sclerites lying just posterior to haptor center. Haptoral tendons were visible. It was identified based on **Yamaguti (1937) ; Whittington *et al.* (2001)** , where can be distinguished from other species by a combination of the following characters: broad body; tiny anterior and posterior hamuli relative to accessory sclerites; all median sclerites located posteriorly on haptor; details of marginal valve; long thin penis; and close proximity of common genital pore and vaginal pore (Figure 4).

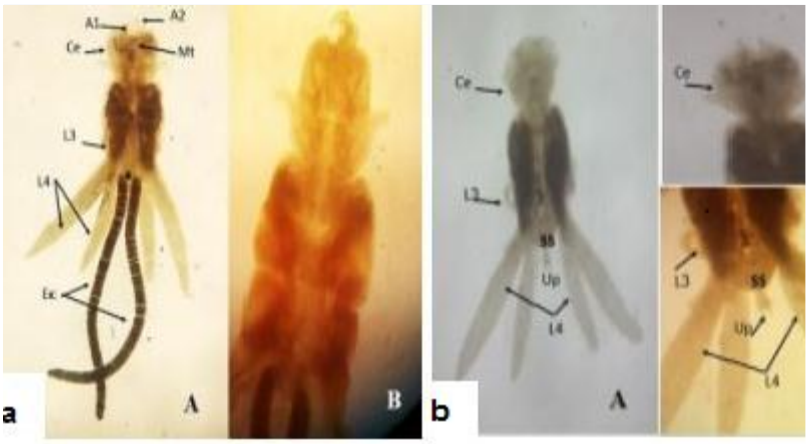


Figure 2: showing the Light photomicrograph of Genus: *Lernanthropus* a: female of *Lernanthropus* sp. b: male of Lernanthropus species.
A: whale body copepod. **B:** lateral view in the photo of female and anterior part in the photo of male **C:** posterior end. **A1:** first antenna; **A2**: second antenna; **Mt:** mouth tube; **M:** maxilliped; **M2**: second maxilla; **Ce:** cephalothorax; **L1**; 1st thoracic leg; **L2**: 2nd leg; **L3**: 3rd leg; **L4:** 4th leg; **a:** abdomen; **SS:** spermatophore sac; **Up:** Uropods; **Ec:** egg sac

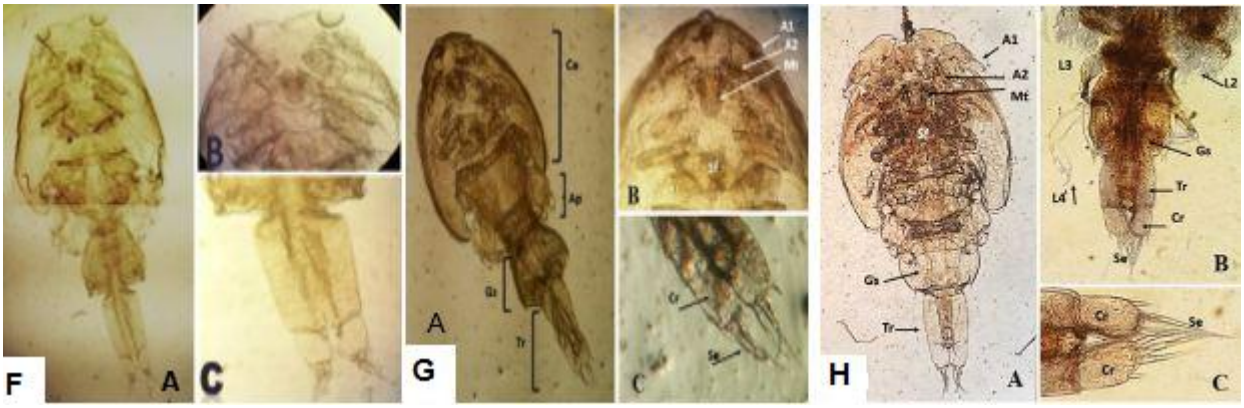


Figure 3: showing the Light photomicrograph of Family: Caligidae F: female of *Caligus lichiae* G : female of *Lepeophtheirus lichiae* H: male of *Lepeophtheirus lichiae* **A:** whole body of copepod, **B:** anterior part of Cephalothorax. **C:** posterior end. Lun: lunule; Fp: Frontal plate; **A1:** first antenna; **A2:** second antenna; Mt: mouth tube; M: maxilliped; **M2:** second maxilla; **Sf:** Sternal furca; **L1**:1st thoracic leg; **L2:** 2nd leg; **L3:** 3rd leg; **L4:** 4th leg; **Es**: egg sac; **Ce**: cephalothorax; **Ap:** Apron; **GS:** genital segment; abd: abdomen; **Cr:** Caudal rami; **S:** spine.

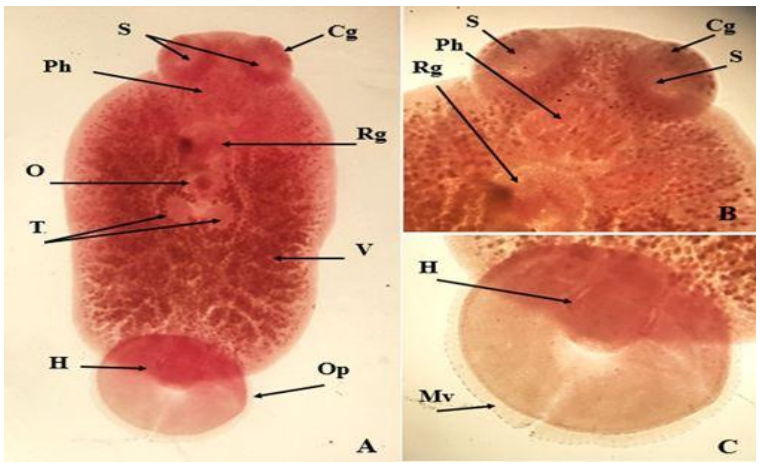


Figure 4: Monogenean parasite isolated from *Moolgarda seheli* A: whole fluke of *Benedenia sekii*
stained with alum carmine **B:** Anterior end. **C:** opisthaptor of *benedenia*.
Cg: Cephalic gland, **S:** Sucker, **Ph:** Pharynx**, Rg:** Region of genitalia**, O:** Ovary, **T:** Testes**, V:** Vetelline glands**, Op:** opisthaptor, **Mv:** Marginal valve**, H:** Hooks.

3.3.Molecular detection of *Benedenia* species (Monogenetic trematodes):

Recognition of *Benedenia* species (family: Capsalidae, monogeneans) used 28S rRNA gene selected the target sequence of C1/D2 primer combinations. The results obtained upon gel electrophoresis analysis of clearly visible bands were detected around 800bp sequence.

3.4. DNA sequence characteristics:

Primer C1/D2 pairs for 28SrRNA generated larger sequence fragments (approx. 800 bp) but because alignment was ambiguous, were excluded from analyses reducing the final number of characters to only approximately 397 bp after removing the gaps which used in analyses for 48 sequence in family Capsalidae. These sequences were selected from GenBank blast result of the *Benedenia* query sample.

3.5. Phylogenetic analyses:

The phylogenetic tree of family capsalidae split into three major clades **(**Figure 5) .The Sequences of C1 28S rRNA of benedenia species taken from Egypt (Ismailia) (sequenced in this study) and recorded in this study showed a close relationship between species that was recorded from NCBI (National Center for Biotechnology Information). The best similarity of benedenia species (query sample) was recorded with Benedenia seki*i* from Australia (FJ971971.1) which was 97.9% followed by 98 bootstrap value and *Benedenia sciaenae* from Australia (FJ971970.1) which was 97.2% followed by 94 bootstrap value were these species in the same genus Benedeniinae. The least similarity was recorded with *Entobdella stenolepis* from Canada (FJ971991.1) which was 86.3% and *Capsala martinieri* from South Africa (FJ971980.1) which was 87.6% were these species in different genera (*Entobdella* and *Capsala*) other than *Benedeniinae* (Table 1) .

3.6. Prevalence and seasonal variations:

As shown in Table 2 ,the total prevalence of infestation was (31%) where the total prevalence of *Lernanthropus* was (24.5%), *Caligus* Species was (5%) and monogenea was (13%). The total prevalence of mixed infestation between crustaceans with monogenea was (7%). The seasonal prevalence of parasitic infestation was mostly highest in winter season and the lowest was summer season as in *Lernanthropus* species Infestation. On other hand, highest infestation of caligus was recorded the highest in the spring season and disappeared in the other seasons and monogenean infestation was highest in the spring season and disappeared in the autumn and summer seasons .

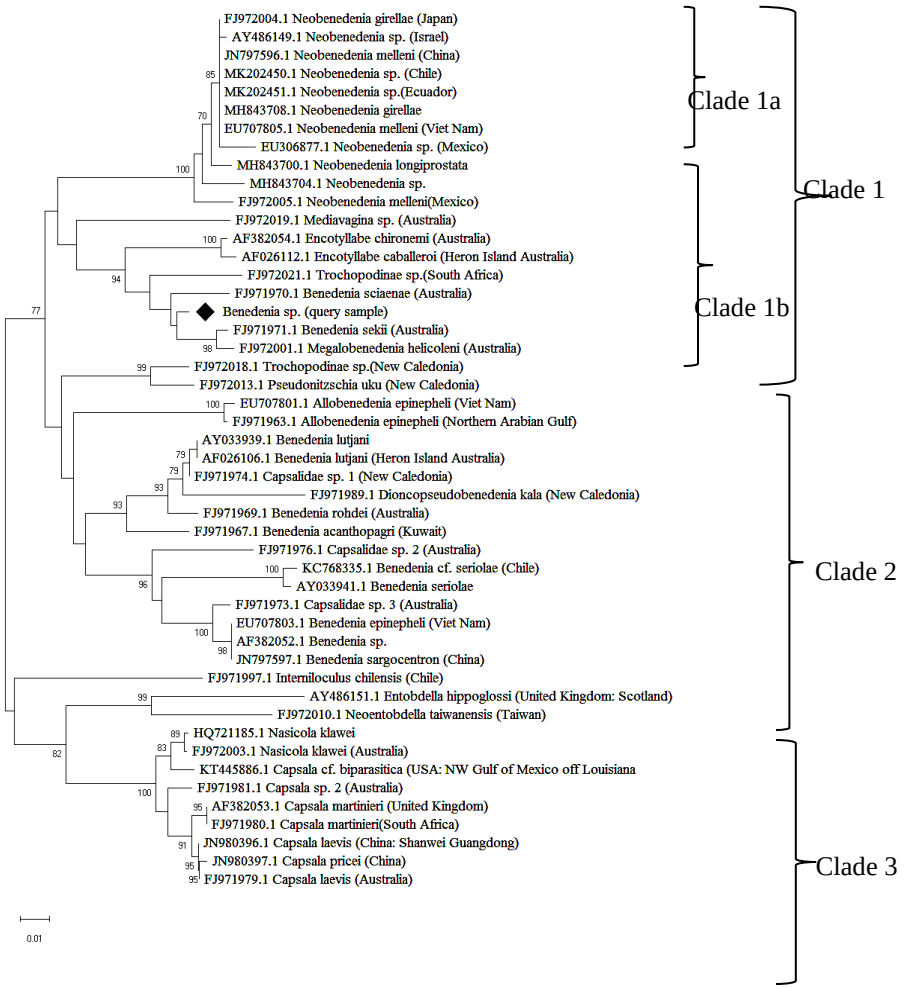


Figure 5:phylogenetic tree based on 28S rRNA of *Benedenia* spp. from Egypt (Ismailia) and other Sequences from GenBank, inferred using the Neighbor-Joining method, Kimura 2-parameter model, supporting with 1000 bootstrap which shown next to the branches. ♦ Sequences of C1 primer of benedenia spp. taken from Egypt (Ismailia).

Table 1:showning Sequence Identity Matrix of the *Benedenia* sp (query sample) (sequenced in this study) with the other sequence from GenBank.

	Seq->	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	Benedenia sp.(Query sample)		94.4%	97.9%	97.2%	94.4%	89.4%	91.6%	90.4%	90.9%	91.1%	90.4%	90.1%	89.9%	87.1%	88.9%	87.6%	86.3%
2	AF026112.1 Encotyllabe caballeroi(Heron Island, Australia)	94.4%		93.1%	93.9%	91.1%	88.1%	89.1%	88.6%	88.4%	89.6%	88.6%	87.4%	88.1%	85.3%	86.9%	85.6%	83.6%
3	FJ971971.1 Benedenia sekii (Australia)	97.9%	93.1%		96.4%	95.9%	89.4%	90.1%	88.9%	91.1%	90.4%	88.9%	89.9%	88.4%	86.1%	87.4%	85.8%	84.6%
4	FJ971970.1 Benedenia sciaenae (Australia)	97.2%	93.9%	96.4%		94.2%	89.6%	90.6%	88.9%	90.6%	89.4%	88.9%	90.1%	88.4%	87.1%	87.9%	86.3%	84.6%
5	FJ972021.1 Trochopodinae sp.(South Africa)	94.4%	91.1%	95.9%	94.2%		87.1%	87.4%	89.6%	88.1%	88.4%	89.6%	87.9%	88.6%	85.8%	87.4%	85.6%	83.8%
6	EU707803.1 Benedenia epinepheli (Viet Nam)	89.4%	88.1%	89.4%	89.6%	87.1%		90.6%	87.9%	93.6%	92.1%	87.9%	92.4%	87.4%	93.4%	86.3%	85.6%	85.3%
7	FJ971963.1 Allobenedenia epinepheli (Northern Arabian Gulf)	91.6%	89.1%	90.1%	90.6%	87.4%	90.6%		88.6%	90.1%	90.6%	88.6%	90.1%	87.9%	89.1%	87.1%	86.6%	87.1%
8	EU707805.1 Neobenedenia melleni (Viet Nam)	90.4%	88.6%	88.9%	88.9%	89.6%	87.9%	88.6%		89.1%	89.4%	100%	89.9%	98.9%	86.1%	87.4%	87.4%	83.8%
9	AF026106.1 Benedenia lutjani (Heron Island, Australia)	90.9%	88.4%	91.1%	90.6%	88.1%	93.6%	90.1%	89.1%		95.7%	89.1%	98.2%	89.1%	90.4%	86.1%	85.6%	84.6%
10	FJ971967.1 Benedenia acanthopagri (Kuwait)	91.1%	89.6%	90.4%	89.4%	88.4%	92.1%	90.6%	89.4%	95.7%		89.4%	94.9%	89.4%	90.4%	87.3%	86.8%	84.8%
11	FJ972004.1 Neobenedenia girellae(Japan)	90.4%	88.6%	88.9%	88.9%	89.6%	87.9%	88.6%	100%	89.1%	89.4%		89.9%	98.9%	86.1%	87.4%	87.4%	83.8%
12	FJ971969.1 Benedenia rohdei (Australia)	90.1%	87.4%	89.9%	90.1%	87.9%	92.4%	90.1%	89.9%	98.2%	94.9%	89.9%		89.9%	89.8%	86.1%	85.1%	83.8%
13	MH843700.1 Neobenedenia longiprostata	89.9%	88.1%	88.4%	88.4%	88.6%	87.4%	87.9%	98.9%	89.1%	89.4%	98.9%	89.9%		85.6%	86.9%	86.9%	83.1%
14	KC768335.1 Benedenia cf. seriolae (Chile)	87.1%	85.3%	86.1%	87.1%	85.8%	93.4%	89.1%	86.1%	90.4%	90.4%	86.1%	89.8%	85.6%		85.6%	85.3%	83.6%
15	FJ972003.1 Nasicola klawei (Australia)	88.9%	86.9%	87.4%	87.9%	87.4%	86.3%	87.1%	87.4%	86.1%	87.3%	87.4%	86.1%	86.9%	85.6%		97.4%	88.6%
16	FJ971980.1 Capsala martinieri (South Africa)	87.6%	85.6%	85.8%	86.3%	85.6%	85.6%	86.6%	87.4%	85.6%	86.8%	87.4%	85.1%	86.9%	85.3%	97.4%		89.1%
17	FJ971991.1 Entobdella stenolepis (Canada)	86.3%	83.6%	84.6%	84.6%	83.8%	85.3%	87.1%	83.8%	84.6%	84.8%	83.8%	83.8%	83.1%	83.6%	88.6%	89.1%	

Table 2: Total and Seasonal prevalence of ectoparasites infestation in examined *Moolgarda seheli*

Season	***Lernanthropus* species**		***Caligus* species**		**Monogenea (*Benedenia* species)**		**Total parasitic infection**	
	Prevalence	Number of infested fishes	Prevalence	Number of infested fishes	Prevalence	Number of infested	Prevalence	Number of infested
Summer (n=30)	10%	3	0%	0	0%	0	10%	3
Autumn (n=100)	12%	12	0%	0	0%	0	12%	12
Winter (n=40)	50%	20	0%	0	32.5%	13	67.5%	27
Spring (n=30)	46.67%	14	33.33%	10	43.33%	13	66.67%	20
Total (n=200)	24.5%	49	5%	10	13%	26	31%	62

n examined fish per season

3.7. Histopathological findings:

As shown in Figure) microscopical examination of the gills of naturally infested Moolgarda seheli with *Lernanthropus* species showed section of parasite (arrows) that causing severe destruction and massive inflammatory reaction (stars). Higher magnification of gills showed cross section of the parasite and severe hyperplasia and leukocytic infiltration of gill lamellae, along with congestion. While gills infested with *Caligus* species showed sever destruction and complete loss of gill lamellae. Gills infested with monogenea species showed severe congestion and hemorrhages at the base of the gill arch as well as the primary lamellae along with destruction of gill lamellae.

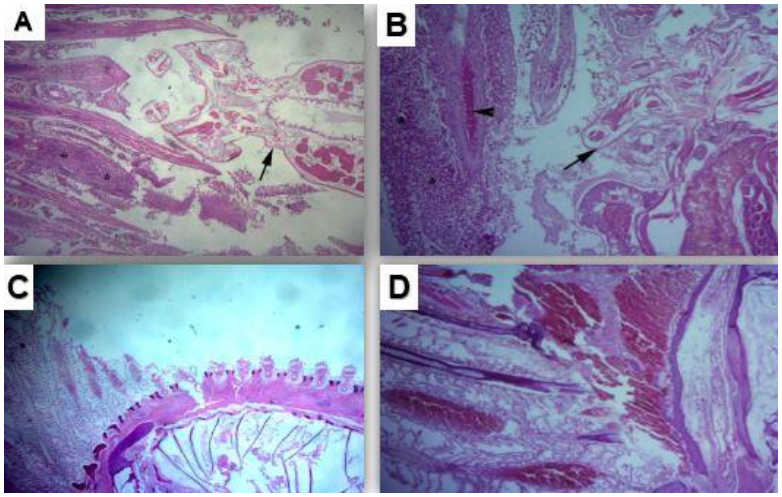


Figure 6: The histopathology of gills of *Moolgarda seheli* **A:** Gills infested with *Lernanthropus* species. showing section of parasite (arrows) that causing severe destruction and massive inflammatory reaction (stars). **B:** Higher magnification of gills infested with *Lernanthropus* species. showing cross section of the parasite and severe hyperplasia and leukocytic infiltration of gill lamellae (arrow heads, along with congestion (arrowhead). **C:** Gills infested with *Caligus* spieces. showing severe destruction and complete loss of gill lamellae. **D:** Gills infested with monogenea sp. showing severe congestion and hemorrhages at the base of the gill arch as well as the primary lamellae along with destruction of gill lamellae, H&E. A&C ,X 40. B&D, X 100.

DISCUSSION

The main clinical sign of naturally infested *Moolgarda seheli* showed no obvious clinical abnormalities except in case of heavy infestation with the copepod crustacean parasites**.** The pervious results agreed with **El-Deen *et al.* (2013), Abdel-Mowla *et al.* (2015) and Eissa *et al.* (2016)** . Regarding the postmortem examination of gills, it revealed marbling appearance with excessive mucus secretion and sticking of gill tips and grayish

discoloration this result agreed with that described by **Osman (2005).** The pervious result could be likened to the severe irritation caused by the parasite's movement and feeding activities. Excessive mucus secretion may also act as defense mechanism by which the host tries desperately to get rid of the irritating parasites. Gill marbling results from blood circulation obstruction in the branch filaments resulting in their destruction as a result of parasite feeding and hypertrophy pressure. This result agreed with **Abdel-Mowla & El-Ekiaby (2012); El-Deen *et al.* (2013); Eissa *et al.* (2017 b) and Qorany (2020)** . *Lernanthropus* species was isolated from the gills of *M. seheli* this agreed with the result obtained by **El-Deen *et al.* (2013) and Hassanin (2016)** who isolated the same parasite from *Mugil cephalus* and *Moolgarda seheli*. *Caligus lichiae* was isolated from the gills of *M. seheli*. The morphological feature was confirmed by **Brian (1906)** . This results was in agreement with that obtained by **Özak *et al.* (2019)** who collected from *Lichia amia* (Linnaeus) and from a second Carangid, *Seriola dumerili* (Risso), where both caught in the Gulf of Iskenderun, Turkey they mentioned that the *C. lichiae* is either extremely rare or has been confused with another species so they declare the write description of this species. *Lepeophtheirus lichiae* was isolated from the gills of *Moolgarda seheli*. The morphological feature was confirmed by **Barnard (1948)** and agreed with the result obtained by **Sakarya *et al.* (2019)** who collected from the dorsal body surface of the leerfish, *Lichia amia* (Linnaeus) caught in North-Eastern Mediterranean waters off the Turkish coast. *Benedenia sekii* (Family: Capsalidae) was isolated from gills and oral cavity of the *M. seheli* range from 2-7 parasites per fish. Its morphological character was agreed with **Sharples & Evans (1995) and Maran *et al.* (2014)** who obtained the same parasite from the body surface of *snapper Pagrus auratus*. Regarding the identification of *Benedenia species* (Family: Capsalidae monogeneans) parasite using PCR, where based on traditional morphological characters (different mainly by possession of an aseptate) Benedeniinae was large subfamilies consist of 13 genera and approximately 51 species, which represent about >25% of Capsalidae diversity **(Whittington, 2004)** . The polyphyly of the Benedeniinae indicate extensively that relationships were widely misunderstood in this subfamily in additional to neobenedenia could be placed in a separate subfamily. The confusion of composition of the Benedeniinae, not realistic for a new subfamily to be built without first reexamining what the subfamily to which Neobenedenia presently belongs (Perkins *et al.*, 2009). Therefore, the molecular methods set out here offer useful instruments for future studies of specimens of various fish species and geographical origins. In this study, molecular identification of *Benedenia* species using PCR analysis of the 28SrRNA (mix of C1/D2 pairs) generated larger sequence fragments which were a common molecular identification. The 28SrRNA (C1/D2 pairs) amplicons at approx. 800 bp were agreed with Chisholm *et al.* (2001)and Perkins *et al.* (2009). In some of the parasite taxas, many genetic studies have found cryptic complexes. (Bray, 2005). In these cases, morphological analyses showed a one parasite species infecting several hosts; however, the genetic information revealed several parasite species showing high host specificity **(Poulin & Keeney, 2008) .** Therefore, partial sequencing and phylogenetics of 28S rRNA gene of C1/D2 pairs isolated from the positive sample with various nucleotide sequences were downloaded from GenBank (NCBI) and aligned with the identified sequences were analyzed. The results revealed that our sample were more closer related to *Benedenia sekii* from Australia (FJ971971.1) which was 97.9% followed by *Benedenia sciaenae* from Australia (FJ971970.1) which was 97.2% were these species in the same

genus *Benedeniinae*, while was far from *Entobdella stenolepis* from Canada (FJ971991.1) and *Capsala martinieri* from South Africa (FJ971980.1) were these species in different genera (*Entobdella* and *Capsala*) other than *Benedeniinae* and this result was agreed with **Perkins *et al.* (2009)** . Concerning the total prevalence of Lernanthropus species infestation of the examined M. seheli for was 24.5%. This result was nearly agreed with the result obtained by **El-Deen *et al.* (2013)** who recorded about 20% and Abouzaid *et al.* (2018) was 18% of the examined sea bass. While this result was higher than that obtained by **Hassanin (2016)** which recorded as 5.14% of *Lernanthropus* species from *M.seheli*. Concerning the total prevalence of *Caligus* Species infestation of the examined *M. seheli* was 5% (*Lepeophtheirus* was 4% *Caligus* was 1%). This result was closer to that obtained by **Jiann Hsiung *et al.* (2001)** which was 5.3% who detected *Caligus* species among the grey mullet, **Abdel-Mowla *et al.* (2015)** which was 5% in the *Scoberomorus commerson* and **Qorany (2020)** which was 2.15% in Sparus aurata. This result was lower than that obtained by **Hassanin (2016)** which recorded as 33.14% of caligus species from *Moolgarda seheli* from different location. This different is due to *Caligus* obtained in this study from one location which was the gills while in the other study collected from gills and skin. Concerning the total monogenetic trematodes (*Benedenia* species) infestation in the examined *M. seheli* was 13%. This result is closer to that obtained by **Eissa *et al.* (2017 a)** which was 22% in the other species. The parasite load (2-7) per fish was observed in wild fishes. This result was closer to that obtained by **Sikkel *et al.* (2009)** which wasn't more than 12% in ocean surgeonfish (*Acanthurus bahianus*). This result was less than obtained by **Jithendran *et al.* (2005)** which was (27%) .This different in the results due to this study was performed in the cultured fish where the high intensity affect the immunity and increase the rate of infestation where, the benedenia species lived naturally on a local teleost species 'switched' to the larger biomass of cultured fish when sea temperatures dropped and perhaps depressed the immunological defenses **(Deveney *et al.*, 2001).** Concerning the total prevalence of the mixed infestation between crustacean and monogenea in the examined fish was 7%. This result was closer to the result of mixed infection recorded by **Abd Al-Galill (2016)** which was 4%, while it was lower than obtained by **Qorany (2020)** which was 23.42% in other fish species. This difference is due to variation of the location were the fish collected, the climate temperature, crowding and fish species with different habitat. Regarding to the seasonal prevalence of *Lernanthropus* infestation in the examined *Moolgarda seheli* was the highest in the winter (50%) followed by spring (46.67%) then autumn (12%), while the lowest was the summer (10%). This result w*as* disagreed with that obtained by **Hassanin (2016)** which recorded the highest season was summer (12%) followed by spring (7%) then autumn (4%) and absent in winter in *M. seheli*. this different because locality difference and the climatic change over year. Regarding to the seasonal prevalence of *Caligus* infestation in examined *M. seheli* was the highest in the spring (33.33%) (*Lepeophtheirus* was 26.67% and *Caligus* species was 6.67% while disappeared in the other seasons. This result was closer to that obtained by El-Deen *et al.* (2013) which recorded the highest season was the spring (90%) followed by summer (71.3%) and disappeared in other season in the *Mugil cephalus* and **Abdel-Mowla *et al.* (2015)** which recorded the highest season was summer (12%) followed by spring (8%) and not present in the other season in other fish species. This result was disagreed with that obtained by **Hassanin (2016)** which recorded the highest season was summer (44%) and the lower was winter (12%) in *M. seheli* this

different because locality difference. Regarding to the seasonal prevalence of monogenean (*Benedenia* species) infestation to the total examined *M. seheli* was the highest in the spring (43.33%) followed by winter (32.5%) then autumn and the summer (0%) was the lowest. This result was agreed with the result recorded by **Qorany (2020)** were the spring was the highest season of the infestation in other fish species. Also, this result was disagreed with **Mohamed *et al.* (2015)** where the highest season of infestation was the summer and the lowest infestation rate was recorded at the winter. This difference could be because of different geographical areas and/or host factors in additional to the nature of benedenia species The cycle of life is affected by the temperature when the heat was higher and salinity higher. In contrast, cool, hypersaline conditions increased the longevity and the success of infection **(Brazenor & Hutson, 2015)** . Temperature is considered an important factor in the control of monogene reproductive and survival rates **(Bakke *et al.*, 2007; Winger *et al.*, 2007; Luo & Yang, 2010).** Some monogene species tend to reproduce more quickly at higher water temperatures, while others favor cool water temperatures **(Ozturk & Altunel, 2006; Luo & Yang, 2010).** Concerning the histopathological change in gills of *M. seheli* naturally infested with *Lernanthropus* species in the revealed severe atrophy of the gill lamellae along with mechanical destruction of some secondary and primary lamellae. Some cases revealed focal infiltrations with leukocytes, in addition to congestion of the gill arch blood vessels, along with congestion. This result agreed with that obtained by **Manera and Dezfuli (2003); Abdel-Mowla *et al.* (2015); Qorany (2020).** Concerning the histopathological change in gills of *M. seheli* naturally infested with Caligus species revealed sever destruction and complete loss of gill lamellae filaments, degeneration and massive atrophy filaments due to attachment and feeding of copepods. This result agreed with that obtained by **Ghobashy (2000); Nike F. Aladetohun *et al.* (2014).**Concerning the histopathological change in gills of *M. seheli* infested with monogenetic trematodes showing branchitis, marked congestion and hemorrhages at the base of the gill arch as well as the primary lamellae along with destruction of gill lamellae. This result agreed with that obtained by **El-Lamie (2007);Abdelmonem *et al.* (2009); Clarke *et al.* (2012); Eissa *et al.* (2017 a)** .

CONCLUSION

We can conclude this work as *Caligus lichiae*, *Lepeophtheirus lichiae* and *Benedenia sekii* consider as new species founded in this area as well as in this fish. The highest infestation of ectoparasites in *Moolgarda seheli* fish was *Lernanthropus* followed by monogenea and the lowest was Caligus Species The highest seasonal prevalence of ectoparasitic parasites was the winter and the lowest was summer. Molecular identification of *Benedenia* species using PCR analysis of the 28SrRNA (mix of C1/D2 pairs) was a useful and confirmed way for solving the problem of identification of this confusing parasite. The partial sequencing and phylogenetics of the positive sample revealed that our sample were closer related to *Benedenia sekii* from Australia followed by *Benedenia sciaenae* from Australia. Therefore, the molecular methods established and give valuable tools for future research of specimens of fish species and geographical origins.

REFERENCES

Abd Al-Galill, E. (2016). Studies on the Prevailing external parasitic diseases in some marine fishes. Ph. D. Fac.Vet. Med. Suez Canal University.

Abdel-Mawla, I. H. and Shalaby, I. N. (2014). Some studies on monogeniasis and its relation to some heavy metals levels in *Scoberomorus commerson* from Red sea. Abbassa Int. J. Aqua. 7(1): 225-248.

Abdel-Mowla, H. ; El-Lamie, M. and Dessouki, A. A. (2015). Investigation on ectoparasitic crustacean diseases in some Red sea fishes and their associated pathological lesions. . BVMJ. 28(1): 301-309.

Abdel-Mowla, H. I. and El-Ekiaby, W. T. (2012). Studies on the parasitic infection among *Morone labrax* (Seabass fish) as bio-indicator of enviromental conditins. Egyption J. for Aqua. 2(2): 1-15.

Abdelmonem, A. A. ; Metwally, M. M. ; Hussein, H. S. and Elsheikha, H. M. (2009). Gross and microscopic pathological changes associated with parasitic infection in European eel (*Anguilla anguilla*, Linnaeus 1758). Parasitology research. 106(2): 463. https://doi.org/10.1007/s00436-009-1688-2

Abouzaid, A. ; Kamal, E. ; Desouky, A. ; Abou-Rawash, A.-R. and Metazoan. (2018). Metazoan parasite fauna of wild sea bass; *Dicentrarchus labrax* (Linnaeus, 1758) in Egypt. https://doi.org/10.7537/marslsj150618.06

Ali, A. ; Mhaisen, F. and Khamees, N. (2015). Checklists of crustaceans of freshwater and marine fishes of Basrah Province, Iraq. Mesopotamian Journal of Marine Science. 30 (1): 1-32.

Amlacker. (1970). Textbook fish diseases. T.F.H.publ., :Neptune City, New Jersy. 117-135.

Antonelli, L. ; Foata, J. ; Quilichini, Y. and Marchand, B. (2016). Influence of season and site location on European cultured sea bass parasites in Corsican fish farms using indicator species analysis (IndVal). Parasitology research. 115(2): 561-568.

Antonelli, L. ; Quilichini, Y. and Marchand, B. (2010). Sparicotyle chrysophrii (Van Beneden and Hesse 1863)(Monogenea: Polyopisthocotylea) parasite of cultured Gilthead sea bream *Sparus aurata* (Linnaeus 1758)(Pisces: Teleostei) from Corsica: ecological and morphological study. Parasitology research. 107(2): 389-398.

Athanassopoulou, F. ; Ragias, V. ; Tavla, J. ; Christofilogiannis, P. and Liberis, N. (2001). Preliminary trials on the efficacy and toxicity of ivermectin against Lernathropus kroyeri Van Beneden, 1851 in cultured sea bass *Dicentrarchus labrax* L. Aquaculture Research. 32(1): 77-79.

Bakke, T. A. ; Cable, J. and Harris, P. D. (2007). The Biology of Gyrodactylid Monogeneans: The "Russian-Doll Killers". In J. R. Baker, R. Muller, & D. Rollinson (Eds.), Advances in Parasitology (Vol. 64, pp. 161-460). Academic Press. andhttps://doi.org/https:// doi.org/ 10.1016/S0065-308X(06)64003-7

Barnard, K. H. (1948). XVIII.—New records and descriptions of new species of parasitic Copepoda from South Africa. Annals and Magazine of Natural History. 1(4): 242-254. https://doi.org/10.1080/00222934808653905

Bayoumy, E. M. (2003). Monogenean parasites infecting some fishes from the red sea in Egypt Cairo University. Faculty of Science.

Bickford, D. ; Lohman, D. J. ; Sodhi, N. S. ; Ng, P. K. ; Meier, R. ; Winker, K. ; Ingram, K. K. and Das, I. (2007). Cryptic species as a window on diversity and conservation. Trends in ecology & evolution. 22(3): 148-155.

Blainville, H. d. (1822). Mémoire sur les lernées (Lernaea, Lin.). Journal de Physique, de Chimie, d'Histoire naturelle et des Arts, Paris. 95372-380.

Boxshall, G. A. and El-Rashidy, H. H. (2009). A review of the Caligus productus species group, with the description of a new species, new synonymies and supplementary descriptions. Zootaxa. 2271(1): 1-26.

Bray, R. A. (2005). Parasite Biodiversity. By R. Poulin and S. Morand, pp. 216. Smithsonian Institution Books, Washington D.C., 2004. ISBN 1 58834 170 4. US$50.00. Parasitology. 131(5): 725-726. https://doi.org/10.1017/ S003118 2005 21908X

Brazenor, A. K. ; Bertozzi, T. ; Miller, T. L. ; Whittington, I. D. and Hutson, K. S. (2018). DNA profiling reveals *Neobenedenia girellae* as the primary parasitic monogenean in global fisheries and aquaculture. Molecular Phylogenetics and Evolution. 129: 130-137.

Brazenor, A. K. and Hutson, K. S. (2015). Effects of temperature and salinity on the life cycle of *Neobenedenia* species (Monogenea: Capsalidae) infecting farmed barramundi (*Lates calcarifer*). Parasitology research. 114(5): 1875-1886. https://doi.org/10. 1007/s00436-015-4375-5

Brian, A. (1906). Copepodi parassiti dei pesci d'Italia (con XXI tavole). R. Istituto Sordomuti. https://www.biodiversitylibrary.org/item/120114

Chaabane, A. ; Justine, J. ; Gey, D. ; Bakenhaster, M. and Neifar, L. (2016). *Pseudohabdosynochus sulamericanus* (Monogenea, Diplectanidae), a parasite of deep sea groupers (*Serranidae*) occurs transatlantically on three congeneric hosts (*Hyporthodus* spp.), one from the Mediterranean Sea and two from the western. Atlantic. Peer J. 4. https://doi.org/https://doi.org/10.7717//peerj.2233. (e2233)

Chisholm, L. A. ; Morgan, J. A. T. ; Adlard, R. D. and Whittington, I. D. (2001). Phylogenetic analysis of the Monocotylidae (Monogenea) inferred from 28S rDNA sequences. Int. J. Parasitol. 311253-1263.

Clarke, E. O. ; Harms, C. A. ; Law, J. M. ; Flowers, J. R. ; Williams, V. N. ; Ring, B. D. ; McGinty, A. S. ; Hopper, M. and Sullivan, C. V. (2012). Clinical and Pathological Effects of the Polyopisthocotylean Monogenean, *Gamacallum macroura* in White Bass. Journal of Aquatic Animal Health. 24(4): 251-257. https://doi.org/10.1080/ 08997659.2012.713889

Detwiler, J. T. ; Bos, D. H. and Minchella, D. J. (2010). Revealing the secret lives of cryptic species: Examining the phylogenetic relationships of echinostome parasites in North America. Molecular phylogenetics and evolution. 55(2): 611-620.

Deveney, M. R. ; Chisholm, L. and Whittington, I. (2001). First published record of the pathogenic monogenean parasite *Neobenedenia melleni* (Capsalidae) from Australia. Diseases of aquatic organisms. 4679-82. https://doi.org/10.3354/ dao 046079

Eissa, I. ; Derwa, H. ; El Lamie, M. ; Dessouki, A. ; Abo Esa, J. and El Raziky, E. (2016 a). Advanced Studies on Monogenean Diseases of *Argyrosomus Regius*, *Dicentrarchus Labrax* and *Epinephelus Aeneus* in Port Said Governorate. Suez Canal Veterinary Medical Journal. SCVMJ. 21(1): 159-162.

Eissa, I. ; Derwa, H. ; El Lamie, M. and Hassanin, D. (2016). Crustacean Infestations in Some Cultured Marine Fishes in Relation Toeconomical Impact. Suez Canal Veterinary Medical Journal. SCVMJ. 21(2): 17-24. https://doi.org/10.21608/ scvmj. 2016.62621

Eissa , I. ; El-lamie, M. ; Aly, S. and Sallam, N. (2017 a). Studies on the Prevailing Parasitic Diseases in Some Marine Fishes. Egyptian Veterinary Medical Society of Parasitology Journal (EVMSPJ). 1364-77. https://doi.org/10.21608/ evmspj. 2017.37769

Eissa, I. A. ; El-Lamie, M. ; Ali, S. and Khaled, N. (2017 b). Studies on Parasitic Crustaceans among Some Cultured Marine Fishes in Ismailia Governorate. Suez Canal Veterinary Medical Journal. SCVMJ. 22(2): 185-205. https://doi. org/ 10.21608 /scvmj.2017.62172

El-Deen, A. I. E. N. ; Mahmoud, A. E. and Hassan, A. H. M. (2013). Field studies of caligus parasitic infections among cultured seabass (*Dicentrarchus labrax*) and mullet (*Mugil cephalus*) in marine fish farms with emphasis on treatment trials. Global Veterinaria. 11(5): 511-520.

El-Lamie, M. M. T. (2007). Studies on the parasitic diseases in some marine fish Suez Canal Univ. Fac. Of. Vet. Med.

Faisal, D. (2008). Studies on some parasitic diseases caused by harmful crustaceans in fish Suez Canal Univ. Fac. of Vet. Med. .

Ghobashy, M. A. (2000). Morphological and Epidemiological studies on marine fishes ectoparasitic crustacean from the red sea Faculty of Science. Suez Canal University.

Hassanin, D. (2016). Studies on prevailing problems affecting culture marine fishes at Port-Said governorate Fac. Of Vet. Med. Suez Canal Univ.

Jiann Hsiung W. ; Jie Lin L. ; Hong Yon C. ; Chentian W. ; ChenHen W. and T., K. (2001). A survey of the diseases in cultured brood grey mullet (*Mugil cephalus* Linnaeus) in the Yun-Chine-Nan area of Taiwan. J. of the Chinese Society of Vet. Science. 27(2): 89-93.

Jithendran K. P. ; Kk, V. ; Alavandi, S. and Kailasam, M. (2005). *Benedenia epinepheli* (Yamaguti 1937), A Monogenean Parasite in Captive Broodstock of Grouper, *Epinephelus tauvina* (Forskal). Asian Fisheries Science. 18.

Jithendran, K. ; Natarajan, M. and Azad, I. (2008). Crustacean parasites and their management in brackishwater fi nfi sh culture.

Kayis, S. ; Ozcelep, T. ; Capkin, E. and Altinok, I. (2009). Protozoan and metazoan parasites of cultured fish in Turkey and their applied treatments.

Kumar, S. ; Stecher, G. ; Li, M. ; Knyaz, C. and Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Molecular Biology and Evolution. 35(6): 1547-1549. https://doi.org/10.1093/molbev/msy096

Langdon, J. and Jones, B. (2002). Design and implementation of health testing protocols for fish with special reference to sample size, test sensitivity and specifity , predictive value and risk. .

Lucky, Z. (1977). Methods for the diagnosis of fish diseases. In. Amerind Publishing Co., Pvt. Ltd. New Delhi, Bombay Calcutta and New York. .

Luo, Y. and Yang, T. (2010). Seasonal population dynamics of the monogeneans *Pseudorhabdosynochus coioidesis* and *P. serrani* on wild versus cultured groupers in Daya Bay, South China Sea. Helminthologia. 47(4): 241-250. 8https://doi.org/https: //doi.org/ 10.2478/s11687-010-0038-3

Manera, M. and Dezfuli, B. S. (2003). *Lernanthropus kroyeri* infections in farmed sea bass *Dicentrarchus labrax*: pathological features. Dis Aquat Organ. 57(1-2): 177-180. https://doi.org/10.3354/dao057177

Maran, B. V. ; Seng, L. T. ; Ohtsuka, S. and Nagasawa, K. (2009). Records of *Caligus* (Crustacea: Copepoda: Caligidae) from marine fish cultured in floating cages in Malaysia with a redescription of the male of *Caligus longipedis* Bassett-Smith, 1898. Zool. Stud. 48(6): 797-807.

Maran, V. B. A. ; Oh, S.-Y. ; Moon, S. Y. ; Soh, H. Y. ; Kim, C.-K. and Myoung, J.-G. (2014). Monogeneans (Platyhelminthes) from marine fishes of Tongyeong, Korea. Journal of Parasitic Diseases. 38(3): 277-285. https://doi.org/10.1007/s12639-012-0232-y

Mohamed, M. ; Osman, H. ; Magendrran, A. ; Al-Shwared, W. and Fita, N. (2015). Infestation of Cage-Cultured Marine Fish with *Benedenia acanthopagri* (Monogenea; Capsalidae) in Eastern Province of Saudi Arabia. Global Veterinaria. 14219-227. https://doi.org/ 10.5829/ idosi.gv.2015.14.02.9329

Moreira, J. ; Luque, J. L. and Šimková, A. (2019). The phylogenetic position of *Anacanthorus* (Monogenea, Dactylogyridae) parasitizing Brazilian serrasalmids (Characiformes) [La position phylogénétique d'Anacanthorus (Monogenea, Dactylogyridae) parasitant les Serrasalmidés brésiliens (Characiformes)]. Parasite (Paris, France). 2644-44. https://doi.org/10.1051 /parasite/2019045

Morris, T. C. and Costello, M. J. (2019). The Biology, Ecology and Societal Importance of Marine Parasites.

Nelson, J. S. (2006). Fishes of the world (4th Ed. ed.). 601Pp.

Nike F. Aladetohun ; Sakiti, N. G. and Babatunde, E. E. (2014). Histopathological changes induced by copepoda parasites infections on the gills of economically important fish mugilidae (*Liza* falcipinnis and *Mugil cephalus*) from Ganvie area of Lac Nokoue, Republic of Benin. . African Journal of Environmental Science and Technology,. 8(2): 743-147.

Osman, M. A. H. (2005). Studies on Monogenasis among fishes. Fac. Vet. Med., Suez Canal Univ.

Özak, A. A. ; Sakarya, Y. ; Yanar, A. ; Özbilen, U. and Boxshall, G. A. (2019). The re-discovery of *Caligus lichiae* Brian, 1906 (Copepoda: Caligidae) parasitic on two carangid fishes in the Mediterranean Sea, and the recognition of *Caligus aesopus* Wilson C. B., 1921 as a junior subjective synonym. Systematic Parasitology. 96(2): 207-232. https://doi.org/10.1007/s11230-019-09841-3

Ozturk, M. and Altunel, F. (2006). Occurrence of Dactylogyrus infection linked to seasonal changes and host fish size on four cyprinid fishes in Lake Manyas, Turkey. Acta zoologica Academiae Scientiarum Hungaricae. 52407-415.

Padial, J. M. ; Miralles, A. ; De la Riva, I. and Vences, M. (2010). The integrative future of taxonomy. Frontiers in zoology. 7(1): 1-14.

Perkins, E. M. ; Donnellan, S. C. ; Bertozzi, T. ; Chisholm, L. A. and Whittington, I. D. (2009). Looks can deceive: Molecular phylogeny of a family of flatworm ectoparasites (Monogenea: Capsalidae) does not reflect current morphological classification. Molecular phylogenetics and evolution. 52(3): 705-714. https://doi.org/https://doi.org/10.1016/ j.ym pev.2009.05.008

Piasecki, W. ; Goodwin, A. E. ; Eiras, J. C. and Nowak, B. F. (2004). Importance of Copepoda in freshwater aquaculture. Zoological studies. 43(2): 193-205.

Poulin, R. and Keeney, D. B. (2008). Host specificity under molecular and experimental scrutiny. Trends Parasitol. 24(1): 24-28. https://doi.org/10.1016/j.pt.2007.10.002

Pritchard, M. H. and Kruse, G. O. W. (1982). The collection and preservation of animal parasites.

Qorany, R. A. (2020). Molecular Studies on Diagnosis of Some Parasitic Diseases in Seabream and Seabass fishes. Suez Canal Univ. Fac. VET. Med.

Roberts, R. J. (2001). Fish pathology (3rd. ed.). W.B. Saunders, Philadelphia, PA.

Sakarya, Y. ; Özak, A. A. and Boxshall, G. A. (2019). The discovery of *Lepeophtheirus lichiae* Barnard, 1948 (Copepoda: Caligidae) parasitic on leerfish, *Lichia amia* (Linnaeus) in the Mediterranean Sea. Systematic Parasitology. 96(7): 603-616. https://doi.org/10.1007/ s11230-019-09874-8

Sepúlveda, F. and González, M. (2019). DNA barcoding evidence for the first recorded transmission of *Neobenedenia* species from wild fish species to *Seriola lalandi* cultured in an open recirculating system on the Coast of Northern Chile. Aquaculture. 501239-246.

Sharples, A. D. and Evans, C. W. (1995). Taxonomy of the metazoan parasites of the snapper *Pagrus auratus* in New Zealand. New Zealand Journal of Zoology. 22(2): 143-161. https://doi.org/10.1080/03014223.1995.9518031

Sikkel, P. C. ; Nemeth, D. ; McCammon, A. and Williams, E. H., Jr. (2009). Habitat and species differences in prevalence and intensity of *Neobenedenia melleni* (Monogenea: Capsalidae) on sympatric Caribbean surgeonfishes (Acanthuridae). J Parasitol. 95(1): 63-68. https://doi.org/10.1645/ge-1645.1

Whittington, I. D. (2004). The capsalidae (Monogenea: Monopisthocotylea): a review of diversity, classification and phylogeny with a note about species complexes. Folia Parasitol (Praha). 51(2-3): 109-122. https://doi.org/10.14411/fp.2004.016

Whittington, I. D. ; Deveney, M. R. and Wyborn, S. J. (2001). A revision of *Benedenia* Diesing, 1858 including a redescription of *B. sciaenae* (van Beneden, 1856) Odhner, 1905 and recognition of *Menziesia Gibson*, 1976 (Monogenea: Capsalidae). Journal of Natural History. 35(5): 663-777. https://doi.org/10.1080/00222930152023090

Winger, A. C. ; Kanck, M. ; Kristoffersen, R. and Knudsen, R. (2007). Seasonal dynamics and persistence of *Gyrodactylus salaris* in two riverine anadromous Arctic charr populations. Environmental Biology of Fishes. 83(1): 117. https://doi.org/10.1007/s10641-007-9274-x

Yamaguti, S. (1937). Studies on the helminth fauna of Japan. Part 19. Fourteen new ectoparasitic trematodes of fishes. Kyoto, Japan.