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Studies on parasitic copepods of economically important fishes using modern techniques

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INTRODUCTION

General Introduction

1- General Introduction:

In the recent years, parasites have been recognized as an important component of global biodiversity and research efforts directed at documenting parasite species have increased **(Poulin and Morand 2004).** The copepods are a common component of the ectoparasite assemblages of all kind of fishes, from all environments and ecosystems **(Boxshall and Halsey 2004).** In the Neotropics, copepods are the second largest parasite group in marine fishes and the third largest group in the freshwater hosts **(Luque and Poulin 2007).**

Copepod family is commonly found on fishes cultured in brackish and fresh water **(Vinoth *et al.*, 2010).** Attachment sites of these copepods were the body surface and gill cavities of the fish **(Venmathi *et al.*, 2009).** Copepods are extremely important in aquatic ecosystems; they can be a food source for small fish, intermediate hosts of fish parasites or fish parasites themselves, and serve as vectors of disease **(Piasecki *et al.*, 2004).** Parasites play a particularly prominent role in aquatic ecosystems where they can dominate the biomass and productivity of the food webs in which they occur **(Kuris *et al.*, 2008).** However, because parasites are often inconspicuous relative to other members of aquatic communities research into the influence of parasites in aquatic environments has been limited to a few well-studied ecosystems **(Lafferty *et al.*, 2008).**

Human activities, including those in or adjacent to aquatic environments, can have a pronounced effect on the emergence of disease including parasites with implications for human health **(Jones *et al.*, 2008)** and wildlife **(Dobson and**

Foufopoulos, 2001). Because anthropogenic activities increasingly alter the distribution and abundance of parasites **(McKenzie 2007, Morand and Krasnov 2010, Polley *et al.*, 2010)** the ability to predict and mitigate the consequences of emergent infections depends, in part, on our understanding of how human activities influence the role of parasites in ecosystems.

Freshwater aquatic ecosystems are extremely susceptible to diseases, oxygen deprivation, and agricultural runoff **(Scheffer 2001)**. Given their importance in providing food, freshwater, and a harbor for biodiversity, preservation of watersheds is particularly important **(Rosi-Marshall and Wallace 2002).**

Parasitic crustaceans found in the classes Copepoda, Branchiura and Malacostraca are invariably ectoparasitic on fishes and have a direct life cycle, often with a considerable period of time spent off the host. Parasitic stages are usually blood feeders on the gills, fins or skin on the host and in large numbers can have serious pathogenic effects **(Lester and Hayward 2006).** Most species of parasitic crustaceans that have been described are copepods, and the majority of copepods are that infest freshwater fishes come from the families Lernaeidae and Ergasilidae **(Lester and Hayward 2006).** Lernaeids or anchor worms have a wide geographic distribution, although the great majorities are from Asia and Africa. There are over 40 species in the genus *Lernaea*. The most well studied, and probably the most widespread species is *Lernaea cyprinacea* **(Bond 2004; Perez-Bote 2005).**

The Ergasilidae is one of the major families of fish parasitising poecilostome copepods, in which only female adults are parasitic, with larval stage and male adults being planktonic. The females usually find and infest their hosts after mating and undergo a metamorphosis in which the adults change their

body shape and increase in size before beginning egg production**(Lester & Hayward 2006)**.

Parasites can act as sensitive, early indicators of the declining health of ponds, lakes, streams, or rivers **(Bhuthimethee *et al.*, 2005).** In particular, ectoparasites in fish reflect changes in the environment, as poor water quality either increases food supply for parasites or decreases the immune system of the fish and their ability to fight parasites **(Bhuthimethee *et al.*, 2005).** The copepods are a common component of the ectoparasite assemblages of all kind of fishes, from all environments and ecosystems **(Boxshall and Halsey 2004).**

The intensification of production in aquaculture often sees abnormalities in fish behaviour, changes in body colouration, lesions, haemorrhages and ulcerations of the cultured fish **(Leong *et al.*, 2006).** Lack of health management measures for that condition could lead to mortality of the cultured fish. Parasitic infestations associated with other diseases have been responsible for many problems in fish environment **(Leong *et al.*, 2006).** Isopoda, Branchiura and Copepoda are the three major groups of parasitic crustaceans commonly found on fish hosts. Of these, parasitic copepods are the most common and cause increasingly serious problem in cultured fishes **(Boxshall and Halsey, 2004; Johnson *et al.*, 2004).**

Generally, crustaceans are highly variable in body form but some general rules apply. All crustaceans have two pairs of antennae, one pair of mandibles and two pairs of maxillae on their heads. Characteristically, they also have a pair of appendages, laterally, on each body segment although sometimes these are reduced or absent from various parts of the body depending on the species. The appendages can be modified in shape to perform special tasks such as swimming, walking, feeding, respiration or copulation.

Copepods exhibit two body plans: the gymnoplean plan in which the body is divided into two tagmata, an anterior prosome and posterior urosome, at the articulation between fifth pedigerous (leg-bearing) and genital segments (referred to as somites by convention), and the podoplean plan in which the prosome and urosome articulate one somite nearer to the head, between the fourth and fifth pedigerous somites . All the parasites conform to the latter type and, though many are profoundly modified, all can be derived from the basic cyclopiform body plan. Cyclopiform copepods are so-called because they resemble the free-living copepod Cyclops in possessing well-defined body segmentation, clear tagmosis and the entire set of limbs. The prosome comprises a cephalosome made up of the five cephalic somites typical of all crustaceans plus the incorporated maxilliped-bearing (first thoracic) somite, and the first to fourth pedigerous somites. The urosome typically comprises the fifth pedigerous, genital and four free abdominal somites. In adult males all these somites are separate but in most females the genital and first abdominal somites secondarily fuse at the final moult, to form a genital double-somite. Podoplean copepods typically carry their eggs in paired egg sacs, which are extruded from the paired genital apertures and carried by the female until ready to hatch. The presence of paired egg sacs is a useful clue to the identity of very transformed copepod parasites that lack any other morphological characteristics **(Boxshall and Halsey 2004)**.

The basic set of appendages comprises five cephalic and seven thoracic limbs, plus the paired caudal rami located on the anal somite. In order from the front the limbs are: antennules, antennae, mandibles, maxillules, maxillae, maxillipeds and first to sixth swimming legs. The ancestral segmentation and setation patterns were hypothesised for all appendages by **(Boxshall and Halsey 2004),** who noted that the dominant evolutionary trend in copepods is

oligomerisation (fusion of body somites and reduction and loss of appendage segments and setal elements). Oligomerisation typically results from progressive reduction and loss, culminating in the extreme simplification exhibited by the terminal branches of several parasitic lineages within the copepods **(**Fig. 1**).**

Crustacean bodies are composed of several segments generally between 16 to 60 segments. The first six segments form the head and the remainders make up the thorax and abdomen. Most crustaceans have a carapace and all have two pairs of antennae but their structures are greatly varied. The larger freshwater forms are very similar to their marine cousins and are easily recognised. The tiny microcrustaceans are harder to recognise and it may be difficult to see their features without a microscope **(Gooderham and Tsyrlin, 2002)**.

Crustaceans can be found in just about all kinds of waters-fast-flowing, still, fresh and saline. They can be found living in the water column, on the bottom of a water body, or among aquatic plants. Some are tolerant of pollution, while other species are intolerant and prefer clean water. Smaller crustaceans can be found in just about every water body. Some of the larger ones are less common or are found only in particular areas of the state. Adult crayfish have been known to roam the banks of creeks for a period of time and to move from one water body to another if conditions become unfavorable (**Martin and Davis, 2001**).

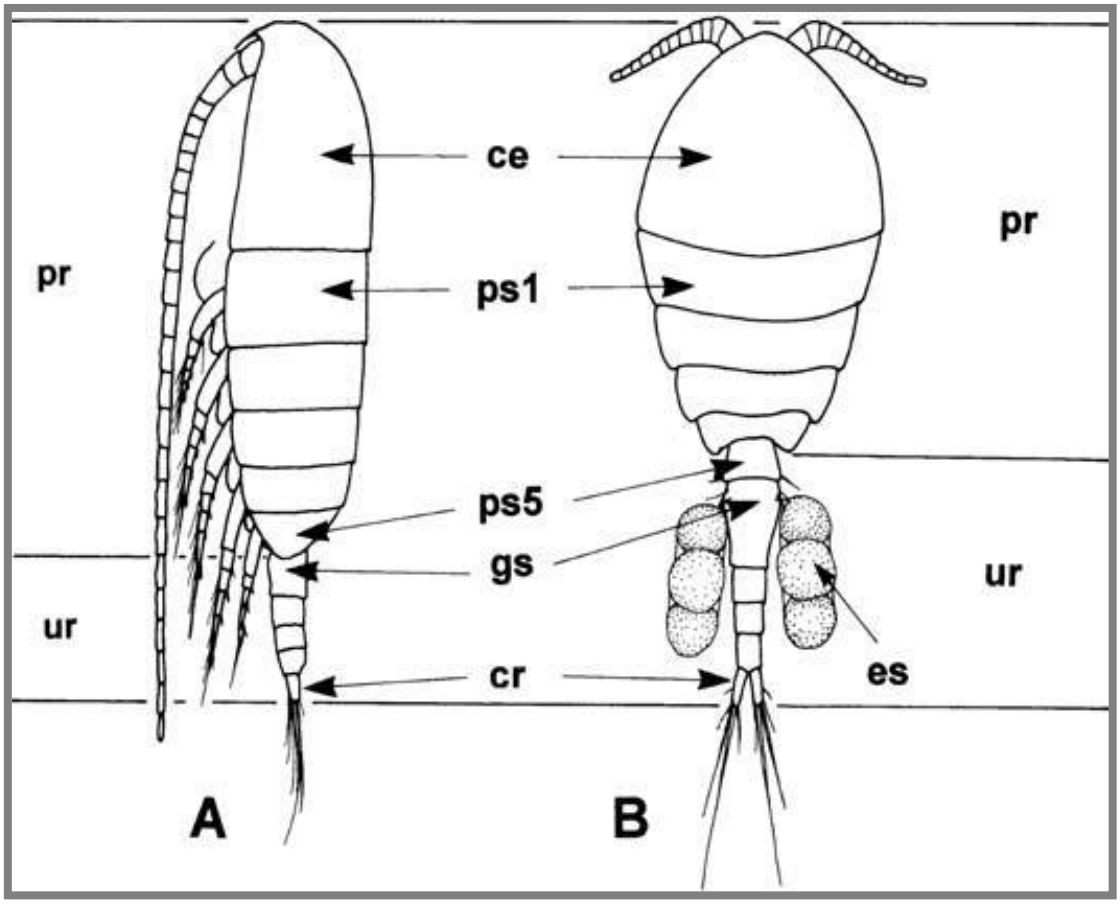

Figure (1): Body plans in the Copepoda. **A**. Gymnoplean plan, showing division between prosome and urosome located posterior to the fifth leg-bearing somite. **B**. Podoplean plan, showing division between prosome and urosome located anterior to the fifth leg-bearing somite. Abbreviations: ce, cephalosome; cr, caudal rami; es, egg sac; gs, genital double-somite; pr, prosome; ps1–5, pedigerous somites 1 to 5; ur, urosome. (redrawn from **Boxshall and Halsey 2004**).

The main parasitic crustaceans of commercial fish belong to the groups Copepoda and Isopoda. Among Copepoda, species of the genera *Argulus, Caligus, Ergasilus, Lernanthropus, Lernaea, Lerneaocera and Lepeophtheirus* (sea lice) parasitize different freshwater and marine fish. They are located on the gills, buccal cavity and skin and produce different degrees of damage, even mortality, depending on the fish species and degree of invasion. Clinical signs include occasional rubbing, decrease of condition and gill damage leading to respiration problems. Inflammed wounds, ulcers and mucous excess can be produced as a consequence of crustacean bites, even affecting muscle. Salmons affected by sea lice may show small white-grey spots. In addition, crustaceans can carry or facilitate other infections.

Although parasitic crustaceans are not as numerous as protozoans or helminths, some of them are important pathogens and diseases caused by them may result in considerable economic losses. The parasitic copepods *Ergasilus sieboldi* or *Lernaea cyprinacea* may provoke veterinary problems in cultures of cyprinid fish whereas *Lernaeocera branchialis* is probably the most serious metazoan parasite of cod *(Gadus morhua)* **(Boxshall, 2004).**

This ectoparasite affects reproduction of infected fish causing delayed gonad development and sexual maturity, and a negative influence on food conversion efficiency, resulting in a significant effect on the condition factor, lower growth ratio in small cod and a loss of weight up to 20–30%, and may cause mortality associated with blood loss, open lesions and possibly occlusion of vessels or aorta. However, the most important group among parasitic crustaceans are undoubtedly sea lice. Another unfavorable impact of copepods on aquaculture facilities is that they may serve as intermediate hosts of important fish parasites such as tapeworms or nematodes. The presence of parasites may lead to fish

mortalities or adversely affect the market value of the fish or fish products when parasites are present in fish muscle. Some important human parasites utilize copepods and fishes as intermediate hosts. A number of parasitic diseases can affect humans, when an infected copepod is accidentally ingested with water.

Parasitic crustaceans sometimes undergo marked modification in external and internal morphology, including partial or complete fusion of body segments, reduction and change of function of appendages, and reduction or disappearance of body cavity organs. Most of these changes enhance the parasitic mode of life. Sensory organs and the reproductive system are usually well-developed. Sexes are usually separate for this parasitic group (dioecious), and sexual dimorphism often is present-especially dwarfism among males **(Hoffman 1999),** (Fig. 2).

The subclass Copepoda has over 10,000 described species **(Huys and Boxshall 1991),** with more than 2000 considered parasitic **(Cressey 1983).** In the transition from free-living to attached parasitic, very distinctive morphologies have developed evolved in copepods. All parasitic copepods have developed appendages for attachment to the host, and specialized mouths, to penetrate the skin and eat the tissue of the host. The siphonostome is the defining characteristic oral cone of the suborder Siphonostomatoida **(Thorell, 1859).** This taxa is composed of 40 families made up of 1550 species, most of which are parasitic on fish **(Huys and Boxshall 1991).** These parasitic copepods are dependent on their hosts, therefor they have evolved complementary biology and life cycles.

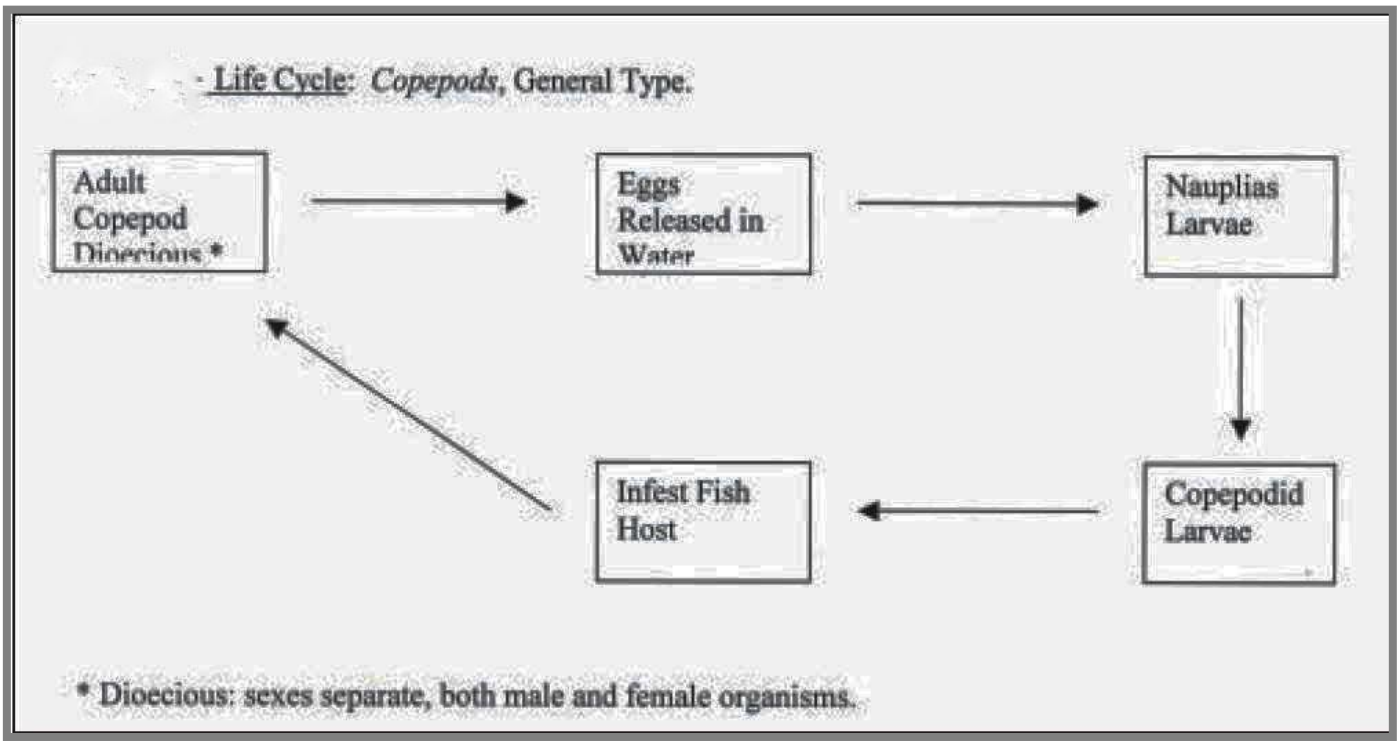

Figure (2): Life cycle of Parasitic copepods. (redrawn from **Hoffman, 1999**).

All species of the suborder Crustacea have naupliar larvae, including parasitic copepods. The number of naupliar stages can vary between parasitic copepod species, along with the number of juvenile attached stages **(Ohtsuka *et al.*, 2009).** Following moults through the naupliar life stages, caligid copepods possess a free-living copepodid stage which allows them to actively seek out their host species followed by moulting on the host and producing a frontal filament for permanent attachment to the host **(Johnson and Albright 1991).** Also many parasitic copepods attach to different areas of the host, and have adapted to their specific microniches **(Tang and Newbound 2004).**

Ectoparasitic copepods which are found on the body of fish hosts are typically flat **(Kabata, 1979a),** such as caligids, while gill parasite morphology is not as restrained by water flow dynamics as evidenced by their different body shapes **(Kabata, 1979a).** Egg production and generation time can also vary greatly between species and is highly sensitive to salinity and temperature **(Ohtsuka *et al.*, 2009).** Generation times range from a few hours between life stages to weeks or longer **(Ohtsuka *et al.*, 2009).** Also egg production is highly variable within caligids ranging from just a few eggs per egg string to hundreds **(Ohtsuka *et al.*, 2009).**

Copepods are the link between primary producers (phytoplankton) and higher trophic levels (fish, birds); they thus play an important role in aquatic ecosystems. The significance of this role has been reported for many planktivorous organisms, including whales **(Baumgartner *et al.*, 2003),** birds **(Karnovsky *et al.*, 2003)** and fish **(Beaugrand *et al.*, 2003).** Since parasitism may reduce copepod fitness even with lethal effect **(Willey *et al.*, 1990, Allen and De Stasio 1993, Chiavelli *et al.*, 1993),** it appears important to assess this phenomenon. The body surface of crustaceans, including copepods, serves as a

convenient living environment for many groups of organisms. These include the so-called epibionts (bacteria, algae and various invertebrates), which settle on the body surface of other living organisms that thereby become basibionts **(e.g., Carman and Dobbs 1997, Fernandez-Leborans and Tato-Porto 2000a,b).** Also, copepods often serve as hosts for parasitic Protozoa, Monogenea or even Isopoda **(Kabata, 1973 and Shields 1994).**

Parasitic copepods can considerable economic losses to both aquaculture and fisheries, and many parasites can be passed to humans through the eating of raw fish **(Bush *et al.*, 2001).** Copepods are a group of 12000 planktonic species of the phylum Crustacea **(Brusca and Brusca, 2003).** Approximately 50% of these species are considered to live in symbiotic associations (including parasitism) with a broad spectrum of aquatic animals, ranging from sponges to marine mammals **(Boxshall, 2005).**

2- Impacts of Parasitic copepods:

1-Mechanical Damage:

I- Fusion of gill lamellae:

Many species of parasites invade the gills of fish. Grossly visible reactions to these parasites on the fish may be non-critical and include a mild discoloration of the gill filaments or one or two white spots. In more critical cases, the fish may display heavy eroding, massive discolorations (often paler), numerous white spots, and increased mucus secretion **(Toksen, 2007).**

Colonization of the gills by parasites often causes proliferative cell changes, including severe epithelial hyperplasia (lamellar gill fusion), hypertrophy, edema, and interlamellar vesicle formation **(Adams and Nowak 2004, Arafa *et al.*, 2009,Taylor *et al.*, 2009).** In the field, this is often recorded as visible white spots,

or white mucoid lesions on the gill surface **(Taylor *et al.*, 2009).** A confirmatory diagnosis cannot generally occur in the field because the presence of microscopic parasites must be confirmed by histological and/or molecular techniques. A method to score gill condition is often used to describe the extent of visible white patches on a scale from negative to heavy infestation **(Taylor *et al.*, 2009).**

These methods are often accurate when there are heavy infestations, but are not accurate with moderate to low infestations when compared to histopathology **(Taylor *et al.*, 2009).** The degree of lesion development is directly proportional to the infective parasite concentration and progression of the infestation **(Morrison *et al.*, 2004, Taylor *et al.*, 2009).**

When individual parasites can be seen with the naked eye, an intensity range is often used to describe the infectivity level within the fish host **(Siquier and Ostrowski de Núñez, 2009).** This number is determined by the quantity of parasites that are found per fish. An intensity range is most useful to compare different species from the same site or the same species from multiple sites **(Siquier and Ostrowski de Núñez, 2009).** Positive correlations between fish length or age and parasite intensity have also been reported. Therefore sampling fish of similar size is important; otherwise results may be skewed with larger fish having more parasites.

II-Tissue Replacement:

Parasite loads in individual fish can often rise to such high numbers that they occupy the majority of the total area of a specific organ. Although tissue replacement is easy to determine, it is one that is frequently ignored in many assessments. Knowledge of a parasite's host response may help researchers more

quickly identify problems within a fish population, emphasizing that parasites should be considered significant pathogens to fish **(Gestel and Azevedo, 2006).**

2- Physiological Damage:

I- Cell Proliferation:

Proliferation of a single type of cell can cause detrimental effects in the fish host. This same proliferation of cell types is found in human diseases such as cancer. For example, carcinogenesis, especially during the initiation and promotion stages, may include interactions between a variety of agents (infectious and chemical). Generalized cell hyperplasia or cellular proliferation, observed in carcinogenesis, is recognized as a causative factor in human liver cancer. Cell proliferation is often caused by the presence of parasites; for example, epithelial cell proliferation is commonly found in Atlantic salmon **(Kania *et al.*, 2010)** and mucous cell hyperplasia has been found in Atlantic halibut **(Otessen *et al.*, 2010).** Parasites are often seen in association with bile duct proliferation in the liver of brown bullheads. Although the relationship between parasites and cancer is rarely studied, these parasites may act as causative agents for carcinogenesis observed in fish species. Although the above statement is speculative, this remains a relatively unresearched topic in fish health.

Amoebic gill disease caused by *Neoparamoeba* sp. in farmed salmon represents another example of parasite-induced cell proliferation. This parasite induces the proliferation of epithelial cells and initiates a hyperplastic response that reduces the surface area available for gaseous exchange **(Villavedra *et al.*, 2005).** The gills of bream (*Abramis brama*) infected with *Ergasilus sieboldi* exhibited hyperplasia and mucus cell proliferation of the respiratory epithelium **(Dezfuli *et al.*, 2003). Dezfuli *et al.*, (2003)** also found that parasitized primary

and secondary lamellae in bream had a higher number of eosinophilic granular cells and rodlet cells. He determined that the increase of inflammatory cells at the site of *E. sieboldi* attachment may be related to an intense host cellular reaction. Rodlet cells represent an inflammatory cell type closely linked to other piscine inflammatory cells (eosinophile granule cells, epithelioid cells, mesothelial cells) **(Dezfuli *et al.*, 2003).**

II- Immunomodulation:

All parasites have evolved ways to evade the host's immune response and host immune systems have evolved numerous ways to counter these evasive strategies **(Sitja-Bobadilla, 2008).** A trade-off is established that is essential to the survival of the parasite and provokes a state of illness in the host, which is not necessarily lethal **(Sitja-Bobadilla, 2008).** However, when a parasite efficiently evades the host immune system, it may damage the host, but actually reduce damage to the parasite **(Sitja-Bobadilla, 2008).**

III- Altered Growth:

Altered growth is perhaps the most difficult mechanism to validate effects due to parasitism. In many studies, researchers have determined that altered growth (delayed growth, stunting) only occurs in extreme laboratory conditions, and would not be observed in the wild **(Karvonen and Sepälä 2008, Tops *et al.*, 2009).** This may be because parasite infested "stunted" fish may not survive in the wild, and they be taken more readily by predation. For the most part, parasites depend on host-derived energy for growth and development, and so they are potentially affected by the host's ability to acquire nutrients under competitive foraging scenarios **(Barber, 2005).** Research by **Barber (2005)** found that the

fastest growing fish developed the largest parasites; therefore, faster growing hosts apparently provide ideal environments for growing parasites.

3- General Biology of Ergasilid Copepods:

1- Morphology of ergasilids:

Members of the class Copepoda are the most numerous of the parasitic crustaceans and are, perhaps, the most common group of parasite crustacean found in fish. Ergasilid copepods are found very often on the gills of several marine and freshwater fish species. In Ergasilidae only the female is parasitic, and is found on the gills of fish. Males are free living and there is a prolonged, free-living larval development which includes three to six stages of nauplii and four to six stages of copepodites (lasting from 10 days to over a month). These free-living stages feed on nanoplankton. Females attached to gills produce eggs in two sacs which are attached to the genital segment. The number of eggs (20-100) is variable with species and apparently also with age and metabolic health as influenced by the location of attachment on the gills. Only subadult and adult females occur of fish, mostly on the gills, a few of the genus *Paraergasilus*, may attach to sites other than gills. The cephalothorax constitutes half or more of body length, the first of four thoracopodes occurs at about midlength. Segmentation of the thorax (except the first segment, fused with the head) and of the abdomen is distinct. The second antenna terminal segment is hooklike in *Ergasilus* and three clawed in Paraergasilus. Eggs are clustered in a bunch rather than arranged in a single line (in caligoids) **(Abowei *et al.*, 2011)**.

Ergasilid copepods are found on the body surface, gills and branchial and nasal cavities of many fish species including sea bass, grouper, mullet, pearl spot, tilapia etc, where it feeds on the blood and epithelium. Heavy infestations can

result in mechanical damage, patechial hemorrhage, impaired respiration, epithelial hyperplasia, and anemia with growth retardation. Severe gill damage is caused by the feeding activity of the copepod and this often leads to fish death. Proliferation of this parasite is observed in summer **(Chivetta, 2012).**

The most common ectoparasitic crustacean on fish is the genus *Ergasilus*. This organism has evolved into a very effective parasite modified for its mode of life. The body attaches to a fish host with prominent claw like second antennae. The cephalothorax or body is highly modified and the body is narrow posteriorly. Females (1.5 to 2.5 mm) are larger than males (1.0 mm). The highly visible egg sacs for the female are long and often cigar-shaped. There are many described species in the world that infest fish. *Ergasilus* attaches to the gill filaments, where it feeds on blood and epithelium. Heavy infestations can result in mechanical damage, petechial hemorrhage, anemia and retarded growth. When the parasite attach, the piscine host becomes more susceptible to secondary infections. Adult parasites occasionally infest gill rakers or other external locations **(Richard, 2003).**

Caligus, *Ergasilus*, are common fresh and marine water parasites that attach to the gill filaments of their host fish. They feed on blood and tissue of the gills causing extensive damage by reducing blood flow and surface area for oxygen exchange. *Ergasilus* are found in many fresh and marine water sources and adversely affect aquaculture, a growing business that helps reduce the overexploitation of wild fish populations **(Piasecki *et al.*, 2004).** *Ergasilus* infections in high number can cause reduce the fitness of the fish and even cause it to die of asphyxia **(Dezfuli *et al.*, 2011).** By understanding the limits and numbers of *Ergasilus* in a fish that cause harm to the host, the reducing *Ergasilus* and their

ability to attach to the gills may be more efficient in preserving the health of fish populations.

The genus *Ergasilus* is the largest genus of ergailids, comprising two thirds of the species currently included in the family **(El-Rashidy and Boxshall 2002).** About 140 species are currently known and most of them are found in freshwater habitats **(Ho *et al*. 1992).** Twelve species of ergasilids are currently known from Australia, with sex species in *Ergasilus* (*E. orientalis, E. australiensis, E. lizae, E. intermedius, E. spinilaminatus and E. ogawai*), three in *Dermoergasilus (D. semicoleus, D.acanthopagri* and *D.amplectens)*, one in *Paraergasilus (P. acanthopagri*) and one in *Neoergasilus (N.spinipes)* **(Kabata 1992).**

2- Pathology of ergasilids:

The gills of fish are tissues specialized for gas exchange, circulation, ion and acid base balance, hormone production, and nitrogenous waste secretion **(Pelster and Bagatto 2010).** Oxygen uptake is driven by diffusion of dissolved oxygen in surrounding water through small plates, called lamellae, with dense capillary networks, and into the blood **(Pelster and Bagatto 2010).** The counter-current exchange system in fish increases oxygen uptake up to five times than in co- current systems. However, ectoparasites of the gill, like *Ergasilus*, may decrease oxygen exchange. For instance, earlier studies have found that fish with high numbers of gill parasites have reduced stamina, spend more time at the water surface, and may increase branching in the gills in order to increase surface area for gas exchange **(Ojha and Hughes 2001).**

In order to compensate for their poor oxygen exchange rates, fish infected with parasites may be pushed into areas with higher dissolved oxygen

concentrations . The presence of *Ergasilus* in different lake environments may have implications about the spread of this ectoparasite.

Infestations of the copepod, *Ergasilus* sp., have rarely been recorded on silver perch farms. Ergasilids are often described as 'gill maggots' due to the appearance of white egg sacs attached to the adult females. The parasite's clasping attachment causes severe gill damage and interference with gill function *Ergasilus*, are common freshwater parasites that attach to the gill filaments of their host fish. They feed on blood and tissue of the gills causing extensive damage by reducing blood flow and surface area for oxygen exchange.

As their name suggests, gill lice attach themselves to the gills of fish and feed on their host's blood and tissue **(Ojha and Hughes 2001).** This attachment causes extensive tissue damage and inflammation, and may render fish susceptible to secondary infection by bacteria, fungi, and viruses **(Dezfuli, *et al.* 2003).** Additionally, the damage to gill tissue can reduce the ability of the fish to maintain normal oxygen uptake by hindering water flow **(Ojha and Hughes 2001).**

Ergasilids attached to gill filaments produce small foci of erosion; apparently feeding involves excretion of proteolytic enzymes for external digestion. Such erosion processes occur in *E. megacheir* infections in cichlids (**Fryer, 1968**) and *E. lizae* infections in grey mullets.

Erosion and degradation processes may extend beyond the epithelial lining, resulting in obstructed branchial blood vessels. Irritation often results in responsive hyperplasia of the epithelium, which, as infection is prolonged, may extend over large areas of the gills, causing fusion and embedding of lamellae, with a resulting decrease in the respiratory function of the gills **(Kabata, 1970; Paperna and Zwerner, 1981).** The nature and magnitude of the pathological

changes in the gills varies with host and ergasilid species. Infection by the more opportunistic species and under stressful conditions (adverse water conditions, deficient nutrition and overcrowding) is likely to induce severe clinical and pathological effects. Marked epithelial hyperplasia is stimulated in the gills of *L. albertianus* infected by *E. kandti*, in a variety of fish infected with *E. cunningtoni* **(Fryer, 1968)** and in tilapia infected with *E. lizae*. Pond reared (70-80 g) *M. cephalus* infested with 100-200 copepods and those of 250 g infested by 1500-2000 copepods were severely emaciated. Losses at harvest reached 50% compared with 10% in uninfested ponds **(Yashuv, 1972; Paperna and Overstreet, 1981).** Emaciation was also observed in wild caught *E. lizae* infected *L. ramada.*

Ergasilids vary in their level of host specificity, some are specific at least to their host genus (notably ergasilids infecting Cichlidae). Others, are less specific in their choice of hosts or are even opportunistic (*E. cunningtoni* has been recorded from fish belonging to seven families, **Fryer, 1968).** However, the least specific and even the opportunistic species (*E. lizae, E. kandti, E. cunningtoni, E. sarsi, Paraergasilus lagoonaris* **(Fryer, 1968; Paperna, 1969; Lahav and Sarig, 1968)** still demonstrate, some predilection for a particular host, while the occurrence in other (not phylogenetically related) fish is either sporadic or linked to particular environmental or stressing circumstances.

The family Ergasilidae (Poecilostomatoida) comprises 24 valid genera (**Amado *et al.*, 1995**) with 249 nominal species **(The World of Copepods 2002).** The overwhelming majority of species occurs in freshwater environments. The morphology of ergasilids largely resembles that of free-living cyclopoids, but some may be extensively transformed, e.g., *Mugilicola*. The best known ergasilids are representatives of the genus *Ergasilus*, which contains 153 nominal species **(The World of Copepods 2002)** and more than 80 valid species **(Kabata 1985).**

The best known species is *Ergasilus sieboldi*, which is 1.7 mm long and attaches to fish gills using its 2nd antennae. The antennae, transformed into powerful hooks, hold the gill filaments tightly and can cause tissue damage and obstruct blood flow. Parasites feeding on epithelial cells stimulate hypertrophy and consequently a coalescence of secondary gill lamellae. This in turn drastically reduces the surface available for gas exchange. Lesions on gills are often attacked by secondary pathogens such as bacteria and fungi. Feeding of *E. sieboldi* was described in detail by **Einszporn (1965a, b).** This particular species attaches to the outside of the gill allowing some of its congeners to explore the space between the gill filaments. In cases of extremely heavy infections of whitefish, the parasite attaches not only to gill filaments but also to the fins **(Kozikowska 1975).**

In Central Europe, the 1st spring generation of *E. sieboldi* becomes sexually mature in mid- June. Their eggs hatch and the copepodids attack fish. They mature and produce a 2nd generation in September. Sometimes a 3rd generation follows by the end of the season. One female can produce 200 offsprings. Theoretically, the ensuing 2nd generation can comprise 40 000 descendants and in the 3rd, as many as 8 million (**Schäperclaus 1992).** *Ergasilus sieboldi* is not host-specific and can infect a majority of freshwater fishes; however the tench, *Tinca tinca*, appears to be the most susceptible. This fact is attributed to the sluggishness of this fish, which may make it more vulnerable to copepod attack. Other less-infected fishes include bream (*Abramis brama*), whitefish (*Coregonus lavaretus*), vendace (*Coregonus albula*), carp (*Cyprinus carpio*), and roach (*Rutilus rutilus*). **Schäperclaus (1954**) described a case of a single, 36-cm-long tench that harbored some 3600 specimens of *E. sieboldi* on its gills. This heavily infected fish had a condition factor of only 0.88. Similarly, intensive infection of peled (*Coregonus peled*) was reported by **Abrosov and Bauer (1961)** from Russia.

Heinemann (1934) found 5431 specimens of *E. sieboldi* on a single tench that died of asphyxia. Severe infection with *E. sieboldi* can result in heavy losses in the yield of tench. In Lake Scharmuzel, Germany, the yield of tench dropped from 5000 kg before the appearance of *E. sieboldi* to 350 kg after its unwanted introduction. In 2 other small German lakes, the yield of tench dropped from 31-47 to 16.5 kg ha-1 after the invasion of this parasite and with copepod prevalences of only 50%. In Lake Grimiz, Germany, the yield of tench between 1926 and 1931 declined from 4583 to 111 kg**,** again a reduction apparently related to *Ergasilus* infections.

3- Life cycle of ergasilids:

In Ergasilidae only the female is parasitic, and is found on the gills of fish. Males are free living and there is a prolonged, free-living larval development which includes three to six stages of nauplii and four to six stages of copepodites (lasting from 10 days to over a month). These free-living stages feed on nanoplankton. Females attached to gills produce eggs in two sacs which are attached to the genital segment. The number of eggs (20-100) is variable with species and apparently also with age and metabolic health as influenced by the location of attachment on the gills. The time required for hatching is temperature dependent, 3-6 days in optimal ambient temperature. High subtropical summer temperatures, coupled with depletion of dissolved oxygen and elevated salinities in lagoon habitats, was shown to be detrimental to both developing eggs and the free living stages of *E. lizae* (syn. *E. nanus*). It has been established that elevated salinity delayed larval development from 10 days at 15 ppt to 27 days at 26 ppt. Biological data on ergasilids infecting fish of African tropical freshwaters are lacking **(Abowei *et al.*, 2011)**.

The life cycle is similar to other copepods. Mature eggs are released from egg sacs. After the eggs are released, embryonic development takes place and a free swimming nauplius hatches from each egg. Larvae pass through four copepodid stages, each accompanied by molting. Copulation occurs when an individual is free swimming, after which the male is free living and the female searching the fish host. The female copepod enters the gill cavity, where is retained by gillrakers, creeps to the gills, and attaches with highly modified appendages. *Ergasilus* is of considerable importance in fishery management, especially for fish inhabiting lakes **(Richard 2003).**

Gill lice, a parasite of the genus *Ergasilus*, is a host-specific ectoparasite that infects many species of freshwater fish including yellow perch, walleye, brook trout, salmon, and large and small mouth bass. Eggs of the gill lice are in external egg sacs attached to the parasitic female. Once developed they hatch and the nauplii, (the first larval stage of many copepods) become free-living. Gill lice of the genus *Ergasilus* go through several copepodid stages (second larval stage) following various nauplii stages. After reaching maturity, adults mate and, while males remain free-living, females become parasitic **(Hudson and Lesko 2003).**

4- General biology of Lernanthropidae:

1- Morphology of Lernanthropidae:

Lernanthropidae **(Kabata 1979b)** is a large family of siphonostomatoid copepods comprising over 150 species. They are exclusively parasitic on gill filaments of marine teleosts. They use their prehensile antennae and maxillipeds to attach tenaciously to a host's gill filaments. The attachment is assisted, in the case of the female, by leg 3 which is modified into a pair of large, folded lamellae designed for clamping onto a host's gill filaments. Thus, lernanthropids can often cause pathological effects like desquamation, erosion, and necrosis of the host's

gill filaments **(Manera and Dezfuli 2003)** and, in cases of heavy infection, may lead to asphyxiation, anemia, and secondary bacterial infections **(Tokşen *et al.*, 2006).**

Lernanthropus De Blainville, 1822, with more than 100 nominal species, is the most species and most widespread genus of the family Lernanthropidae and is considered to be a common genus of parasitic copepods on fishes **(Kabata, 1993).** Lernanthropids are largely parasites of warm-water fishes. Thus, while 44 species are known from India **(Pillai 1985),** only 9 species are known from Japan **(Ho and Do 1985).** So far, the authors have discovered and reported 12 species of lernanthropids from Taiwan **(Ho *et al.*, 2008, Liu *et al.* 2009a, b).**

Lernanthropus is among the most common genera of parasitic copepods, and all species are parasitic on the gills of marine teleosts, most of them inhabiting warmer waters. The genus *Lernanthropus* (Copepoda: Lernanthropidae) consists of more than 100 species of gill parasites and some species such as *Lernanthropus kroyeri* can cause high mortalities in smaller-sized European sea bass *Dicentrarchus labrax* **(Kabata, 1979b; Ho and Do, 1985; Athanassopoulou *et al.*, 2001; Henry e*t al.*, 2009).** As compared to other copepods infecting gills, copepods such as Lernanthropids are larger in size and can be seen with the naked eye. It usually feeds on the gill tissues and blood of its host which can seriously damage host tissues **(Kinne, 1984).**

Lernanthropus kroyeri has been recorded from many localities along the coast of Europe, from the Adriatic Sea to the southern North Sea **(Kabata, 1979b).** It was reported from Aegean Sea, but not from the Black Sea **(Öktener, 2009).** Morphology, histopathology, treatment of *Lernanthropus kroyeri* in cultured Sea Bass have been studied by **(Özel *et al.*, 2004;Korun and Tepecik**

2005; Tokşen *et al.*, 2008). Studies on geographical and seasonality distribution of the parasitic copepods are scanty in Turkey.

The only Lernanthropids previously reported from Turkish waters are *Lernanthropus mugilis* on *Liza aurata* from the Aegean Sea **(Altunel, 1983)**, *Lernanthropus kroyeri* on *Dicentrarchus labrax* from the Aegean Sea **(Özel *et al.*, 2004;Tokşen *et al.*, 2008)**, *Lernanthropus trachuri* on *Trachurus mediterraneus* from the Sea of Marmara **(Öktener, 2004),** *Mitrapus oblongus* on *Sardinella aurita* from the Mediterranean Sea **(Romero and Öktener 2010).**

This species is relatively large, reddish in colour, firmly attached to the gills, inflicts serious damage to the gills by way of erosion, desquamation and necrosis of the secondary lamellae near the site of attachment. The grasping action of the mandibles and the maxillae results in the exposure of blood vessels and hemorrhages. This serious pathogen is frequently encountered in many species of wild fish and cage cultured sea bass.

The body of female is elongated. Body surface ventrally ornamented with patches of setules, cephalothorax with dorsal shield slightly narrower anteriorly, anterolateral corners more rounded than posterolateral corners in dorsal view. Deep constriction between cephalothorax and pregenital trunk. Latter with prominent, rounded anterolateral corners and slightly convex lateral margins. Dorsal plate of fourth pedigerous somite well delimited from third legs, broader posteriorly, in some specimens with posteromedian notch, often somewhat asymmetrical. Genito-abdominal tagma small, with abdomen not distinctly delimited, subquandrangular **(Boxshall 2005).**

Cephalothorax subrectangular, angles rounded, little longer than wide. Genital segment very small, subovate, partly hidden in ventral view by third pereiopods, which were not dissected off to reveal this segment. Abdomen

subrectangular, posterior angles rounded and narrowing slightly posteriorly. Caudal rami is widest at base, narrowing slightly distally, distal margin rounded, three small spines borne distally. Egg sac long, uniseriate, with numerous eggs (Fig. 3), **(Boxshall 2005).**

2- Pathology of Lernanthropidae:

Lernanthropus is known to cause some pathological effects on its host such as erosion, desquamation and necrosis of the secondary lamellae near the site of copepod attachment and the terminal claw of the second antennae lacerated tissue and vessels of infected gill **(Manera and Dezfuli 2003)**. Usually, the parasites cause only minor harm to their hosts when present in small numbers. However, heavy infections can cause severe damage to gill tissues and respiratory impairment accompanied by secondary infections and result in stress and osmoregulatory failure. **Toksen (2007)** reported some symptoms in cultured *Dicentrarchus labrax* infested with *Lernanthropus kroyeri* in the gill show signs of respiratory distress, increase in mucus production and swim in surface water.

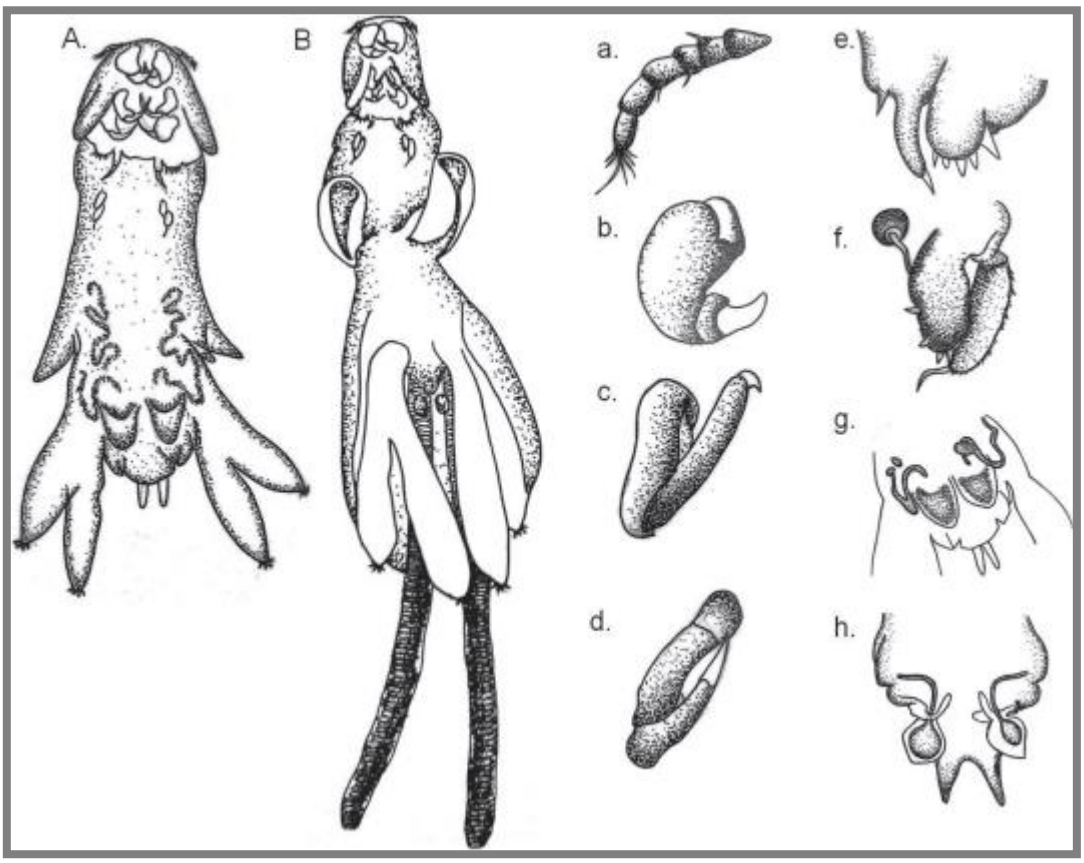

Figure (3): Schematic drawing of *Lernanthropus* sp. **A.** Male, ventral view**; B**. Female, ventral view; **a.** antennule; **b.** antenna; **c.** maxilla; **d.** maxilliped; **e.** leg 1; **f.** leg 2; **g.** posterior extremity of male, ventral view; **h.** posterior extremity of female, ventral view. (redrawn from **Boxshall, 2005**).

Lamellar necrosis, anaemia and secondary infection bacterial infection have also been reported in European sea bass infested with *Lernanthropus kroyeri* **(Manera and Dezfuli, 2003).**

Some species of *Lernanthropus* are strictly host specific, whereas many are parasitic on several species of fish within one or several genera **(Sharp *et al.*, 2003).** Infection of *Lernanthropus* species have been reported in marine teleosts and cultured species such as *Mola mola* in the Gulf of Thailand **(Humphrey *et al.*, 2006),** *Dicentrarchus labrax* in Australia **(Sharp *et al.*, 2003)** and yellowtail kingfish *Seriola lalandi lalandi* in New Zealand **(Ho and Kim, 2004).** In Malaysia, infestation by this group of parasitic copepods (Lernanthropidae) was observed in cage cultured sea bass **(The World of Copepods 2002)**. **(Bahri *et al.*, 2002)** searched to use of *Lernanthropus kroyeri* as a bioindicator of *Dicentrarchus labrax* and *Dicentrarchus punctatus* in Tunisian Inshore Areas.

Lernanthropus sp. has not host specific although some other species are strictly host specific **(Sharp *et al.*, 2003).** *Lernanthropus* is known to cause such pathological changes as, necrosis in epithelial tissue and ligament, increased mucus secretion, narrowing in capillary veins meanwhile *Lernanthropus* attaches to the gill filaments with third leg. *Lernanthropus* species attach to the host gill by means of the piercing action of the antennae, which are assisted by the maxillipeds and the modified third legs **(Kabata, 1993).**

This copepod might be considered as an indicator species, therefore they should be examined for indicator species or seasonal and geographical distribution such as *Dicentrarchus labrax* in Turkey **(The World of Copepods 2002)**. The intensification of production in aquaculture often sees abnormalities in fish behaviour, changes in body colouration, lesions, haemorrhages and ulcerations of

the cultured fish **(Leong *et al.*, 2006).** Lack of health management measures for that condition could lead to mortality of the cultured fish. Parasitic infestations associated with other diseases have been responsible for many problems in fish environment **(Leong, 1994؛ Leong *et al.*, 2006).**

Lernanthropus is known to cause some pathological effects on its host. It attaches to the gill filaments of its host using antennae and third legs and pathological effects such as erosion, desquamation, necrosis in branchial epithelial tissue, increase of mucus secretion, narrowing in capillary veins have been reported **(Manera and Dezfuli 2003** and **Toksen 2007).**

3- Life cycle of Lernanthropidae:

The life cycle of *Lernanthropus* copepod (Siphonostomatoida) comprises two nauplii, one infective copepodid, four fixed copepodids and one preadult. All the attached stages are characterized by the lack of the frontal filament. This is the first description of a life cycle lacking a chalimus for Siphonostomatoida. Siphonostomatoid copepods generally have an infective copepodid stage followed by several attached copepod/chalimus stages prior to maturation to adults. Only a handful of siphonostomes have had their complete life cycles described, out of the 1550 species which make up the suborder. In the extensively researched Caligidae family only 15 of the more than 450 species have complete life cycles described **(Boxshall 2008).**

5 - Economic importance of parasitic copepods:

Parasitic crustaceans serve as both hosts and vectors of viruses as well as of parasites and other microbial pathogenic agents. Few of the presumably numerous associations are known, but many can be anticipated. Recently, branchiurans and

gnathiid isopods have been documented to host helminths and blood parasites Because the agents can be observed readily with a microscope, these are better recognized than are the smaller viral, bacterial, and fungal agents **(Robin 2009).**

There are three main groups of parasitic crustaceans affecting commercially important aquaculture species, most of which are external parasites: the Branchiura, Copepoda and Isopoda. Members of the Branchiura and Isopoda are relatively large and both sexes are parasitic, while copepods, the most common crustacean parasites, are generally small to microscopic with both free living and parasitic stages in their life cycle. Male parasitic copepods die after copulation in the pre-adult stages, so those that are seen attached to fish are generally mature females with distinctive paired egg sacs at the posterior end. The crustacean parasites dealt here are primarily those that are likely to cause problems when commonly cultured fish species are grown in inland low saline or freshwater, though such studies are scarce in India. Under culture conditions, modified specificity is also exhibited by many crustacean parasites in that they will invade 'unnatural' hosts that are not normally present in their natural habitats **(Jithendran *et al.*, 2008).**

Parasitic crustaceans are among the most harmful parasites of fishes. Certain species cause disease outbreaks and mortalities in aquaculture, facilities, and sometimes in natural systems, resulting in serious economic losses **(Lopez 2001).** Fish culture now includes raising fish not only in man-made ponds and tanks, but also in cages and pens built or suspended in large bodies of water such as reservoirs, lakes, and even in marine coastal areas. The practice of introducing fish into large water bodies facilitates transfer of parasites from the introduced fish to the wild populations, and vice versa.

Parasitic copepods introduced into the water body with fish introductions may become established in the natural populations of the water body. Measures recommended eradicating the parasites in ponds or tanks are often inapplicable or impractical in large water bodies. To protect large water bodies against dangerous parasites, entry of the parasites into the habitat must be prevented. In culture systems, high population density is among the most important factors facilitating disease outbreaks. When environmental conditions become favorable for the mass reproduction of the parasite, the disease may spread very quickly from one individual to another. This is particularly true of parasites with direct life cycles such as ectoparasitic protozoans, helminths and crustaceans. Parasites naturally occurring in the lake can infect fish in cages and pens resulting to mass mortality and substantial economic losses. Knowledge of the parasites occurring in fishes both in aquaculture and in natural systems is therefore important for effective management of our fishery resources. The importance of parasites, their use as environmental indicators, controlling and avoiding fish parasites and the effects of fish parasites on humans **(Lopez, 2001).**

Copepods are important vectors of pathogenic helminths, acanthocephalans, bacteria and fungi of fish. A considerable number of copepod species are well adapted for a parasitic mode of life and those that infect fish can cause considerable damage to the host. In spite the substantial economic importance of these parasitic copepods, they have been neglected by workers for quite long time. Although most species of parasitic copepods are parasites on the surfaces, the more economically important are those that penetrate the surface for feeding or attachment **(Cressey, 1983).** The life cycle of parasitic copepods includes free living stages, which undergo a series of molts, followed by maturation and mating; the males then die and the females reach an infective stage and settle on the host.

The family Ergasilidae (Poecilostomatoida) comprises 24 valid genera with 249 nominal species **(The World of Copepods 2002).** The best known ergasilids are representatives of the genus *Ergasilus*, which contains 153 nominal species **(The World of Copepods 2002)** and more than 80 valid species. The best known species is *Ergasilus sieboldi*, which is 1.7 mm long and attaches to fish gills using its 2nd antennae. The antennae, transformed into powerful hooks, hold the gill filaments tightly and can cause tissue damage and obstruct blood flow. Parasites feeding on epithelial cells stimulate hypertrophy and consequently a coalescence of secondary gill lamellae. This in turn drastically reduces the surface available for gas exchange. Lesions on gills are often attacked by secondary pathogens such as bacteria and fungi **(Piasecki *et al.*, 2004).**

In cases of extremely heavy infections of whitefish, the parasite attaches not only to gill filaments but also to the fins. The life cycle comprises 6 nauplius stages, 5 copepodid stages, and adults. Males die after copulation, while females remained attached to the fish host **(Piasecki *et al.*, 2004).** Most ergasilids infest the gills of their host and may cause extensive gill damage and severe hemorrhage, with inflammation and epithelial hyperplasia associated with the attachment and feeding of the parasite **(Lester and Hayward 2006).**

Ergasilid copepods are found on the body surface, gills and branchial and nasal cavities of many fish species including sea bass, grouper, mullet, pearl spot, tilapia etc, where it feeds on the blood and epithelium. Heavy infestations can result in mechanical damage, patechial hemorrhage, impaired respiration, epithelial hyperplasia, and anemia with growth retardation. Severe gill damage is caused by the feeding activity of the copepod and this often leads to fish death. Proliferation of this parasite is observed in summer **(Jithendran *et al.*, 2008).**

The family Ergasilidae comprises a large number of freshwater and occasionally marine parasitic copepods of the suborder Poecilostomatoida. Ergasilids are essentially found on the gills, fins and the nasal cavities of the host fish **(Fryer, 1988)** and in many instances infestation of ergasilids on the gills of freshwater fishes seriously depleted population of desirable fish or caused serious weight loss reducing productivity. *Ergasilus*, being a remarkably successful parasite, have an essentially worldwide distribution **(Fryer, 1988)**. *E. sieboldi*, which is found not only on freshwater fish but also in brackish waters is characterized by a broad specificity having been reported from many different fish families including the Salmonidae, Cyprinidae, Esocidae, Percidae, Siluridae, Cichlidae, Bagaridae, Belonidae, Characidae, Centrachidae, Sparidae and Clupeidae. Since the *Ergasilus* females attack the gills of fish, a heavy infestation can cause severe damage and secondary infections, interfere with respiration, and sometimes kill the host.

In some fisheries and aquacultural enterprises the mortality and morbidity among fish stocks can present serious economic and ecological problems **(Roberts, 2005).** *E. sieboldi* is common on many species of freshwater fish in Europe, but had not been recorded in the British Isles until 1967 on brown. However, the original source of infection was thought to be bream and tench from Stambridge, Essex. **Fryer and Andrews (1988)** also reported *E. briani* from bream in Yorkshire as a second species found on freshwater fish in Britain. In Europe *E. sieboldi* and *E. briani* are serious pests of tench and bream respectively. *Neoergasilus japonicus* is another freshwater ergasilid believed to be introduced to Europe from the Far East and has been reported from the continent only during the late sixties **(Fryer, 1988).**

Another important group of copepod parasites is Lernanthropids that are larger in size and can be seen with the naked eye. It usually feeds on the gill tissues and blood of its host which can seriously damage host tissues and normally they cause only minor harm to their hosts when present in small numbers. However, heavy infections can cause severe damage to gill tissues and respiratory impairment accompanied by secondary infections and result in stress and osmoregulatory failure **(Beng *et al.*, 2012).**

Lernanthropids has a distinct sexual dimorphism in body size and shape. The female's head is fused with the first thorax segment to a pyriform cephalothorax and a dorsal carapace with ventrally turned margins. The other segments are fused with the genital segment covered by a backwards prolonged dorsal plate. The cephalothorax of the males contains the first segment and all other thoracic and genital segments are fused into an elongate trunk **(Harry, 2006).**

Parasitic copepods are common on cultured and wild marine finfish, and there is a substantive literature describing their taxonomy, life cycles, and host ranges. Although many species have long been recognized to have the potential to affect the growth, fecundity, and survival of their hosts, it has only been with the development of semi-intensive and intensive aquaculture that their importance as disease-causing agents has become evident**(Stewart *et al.* 2004).**

All ectoparasitic copepods are known to feed on the blood and tissue fluids of the host. Sites of parasite attachment commonly become haemorrhagic, spongy and necrotic. We also provide a brief review of the environmental and husbandry factors that may affect parasitic copepod abundance and the potential roles that parasitic copepods play as vectors for other disease agents **(Stewart *et al.* 2004).**

Parasites, causing little apparent damage in feral fish populations, may become causative agents of diseases of great importance in farmed fish, leading to pathological changes, decrease of fitness or reduction of the market value of fish. Despite considerable progress in fish parasitology in the last decades, major gaps still exist in the knowledge of taxonomy, biology, epizootiology and control of fish parasites **(Scholz, 1999).** Fish are an indispensable source of proteins for humans, notwithstanding their importance as an object of sport fishery and pets in the case of ornamental fish. Development of aquaculture during the last decades has resulted in much greater attention being paid to problems posed by parasites and their importance for fishery leading to constraints in the productivity of aquaculture **(Scholz, 1999).** Besides direct losses caused by mortality، parasites may have considerable impact on growth and behaviour of fish, their resistance to other stressing factors, susceptibility to predation, etc.; their presence may also reduce marketability of fish **(Kumaraguru *et al*., 1995; Woo, 1995).**

Although parasitic crustaceans are not as numerous as protozoans or helminths, some of them are important pathogens and diseases caused by them may result in considerable economic losses. The parasitic copepods *Ergasilus sieboldi* or *Lernanthropus latis* may provoke veterinary problems in cultures of cyprinid fish whereas *Lernaeocera branchialis* is probably the most serious metazoan parasite of cod (*Gadus morhua*) **(Boxhall and Defaye, 1993; Berland, 1997).**

Parasitic crustaceans are numerous and have worldwide distribution in fresh, brackish and salt waters. Usually they cause only minor harm to their hosts when present in small numbers. However, in case of heavy infections severe damage to skin, muscles, and gill tissue accompanied with secondary infections

can occur. Parasitic crustaceans a great diversity of forms with marked structural modifications to suit their parasitic mode of life **(Jithendran *et al.* 2008).**

Copepods comprise the largest group of crustacean parasites on fish causing economical loss. Disease outbreaks and subsequent mortalities are rare under effective broodstock management systems due to effective treatment methods. However, increasing incidence of copepod parasitism is becoming a regular phenomenon in culture conditions. The only sure way to prevent parasitic infection is to deny the parasite access to the protected habitat.

AIM OF THE WORK

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The Mediterranean Sea fishes especially in coastal waters of Egypt are considered as highly valued fish food. Egypt with its dense and fast growing population, concentrated along the River and Delta region, considers that fish and fishery products play an important role in the country's food security and domestic economy. The aquaculture industry in Egypt is growing rapidly and is currently a top 10 worldwide producer **(FAO, 2005).**

Dicentrarchus labrax (Sea bass) is the most important commercial marine fish species in Egypt and it is commonly used in aquaculture. *Dicentrarchus labrax* is an euryhaline fish **(Monira *et al.*, 1992)** and for this reason, it is important for marine and brackish water fish farming. Moreover, sea bass is an economically important cultured fish species in the Mediterranean coastal waters. The sea bass, *Dicentrarchus labrax* is a demersal species found throughout the Mediterranean Sea and Eastern North Atlantic from Southern Morocco to the Norwegian littoral **(Fritsch *et al.*, 2006).**

Furthermore, In Egypt, mullet fishes especially *Mugil cephalus* are economically very important fish because they have high market value and have been cultivated successfully by fish farmers.

However, the economic impact of parasitic copepods infestation and its pathology on economic marine fishes in Damietta province. The current study was showing the morphological and infection study of parasitic copepods on the gills of *Dicentrarchus labrax* and *Mugil cephalus*. Hence, the present study aims to investigate the pathological changes of parasitic copepods (Ergasilids and Lernanthropidae) based on scanning electron microscopy, transmission electron microscopy and light microscopy.

Therefore, this work is planed to study parasitic copepods infesting some economic marine fishes specially (*Dicentrarchus labrax* and *Mugil cephalus*) caught from the Mediterranean Sea, Damietta City, Damietta Province. Moreover, it is designed to add some information to the database of Egyptian parasitic fauna specially copepods and to investigate:

1- The morphological and anatomical features using modern techniques (electron microscope studies) of collected parasitic copepods in order to highlight adapted criteria developed the parasite to describe the structures enable to play a role in the survival of the copepods parasite.

2- The histological characters of the examined fishes in order to morphologically clarify the fine structures of the host gills using electron microscope studies.

3- Aspects of the pathology and infection caused by parasitic copepods using electron microscope studies.

MATERIALS AND METHODS

Materials and Methods

1- Fish collection:

Marin fishes; sea bass, *Dicentrarchus labrax* and flathead grey mullet, *Mugil cephalus* were collected from Damietta port during summer 2013 (Fig. 4). In total, 130 marine fishes of *Dicentrarchus labrax* and *Mugil cephalus* were transported back to Damietta University, Science faculty for studying. Their body weights and lengths were ranged from less than 50 up to 300 gm and 15 to 35 cm, respectively. The gills from each fish were removed, placed in a petri dish and examined under dissecting microscope. Fish hosts (Fig. 5) are belonging to two families were identified throughout the present investigation depending on **Wheeler (1985)** and **Ibrahim and Soliman (1996)**.

2- Copepod parasites collection:

Body surface, mouth and gills were examined under light and stereo microscope for parasitic copepods. The copepods were removed from the gills of fish using fine needles and a dissecting microscope. Gill filaments with attached copepods were removed.

3- Identification of copepod parasites:

Copepods were identified based on morphological features under light and scanning electron microscopes (SEM). Some specimens were fixed in 10% formaldehyde. They were washed under running tap water over-night to remove formalin then dehydrated in ascending series of alcohol.

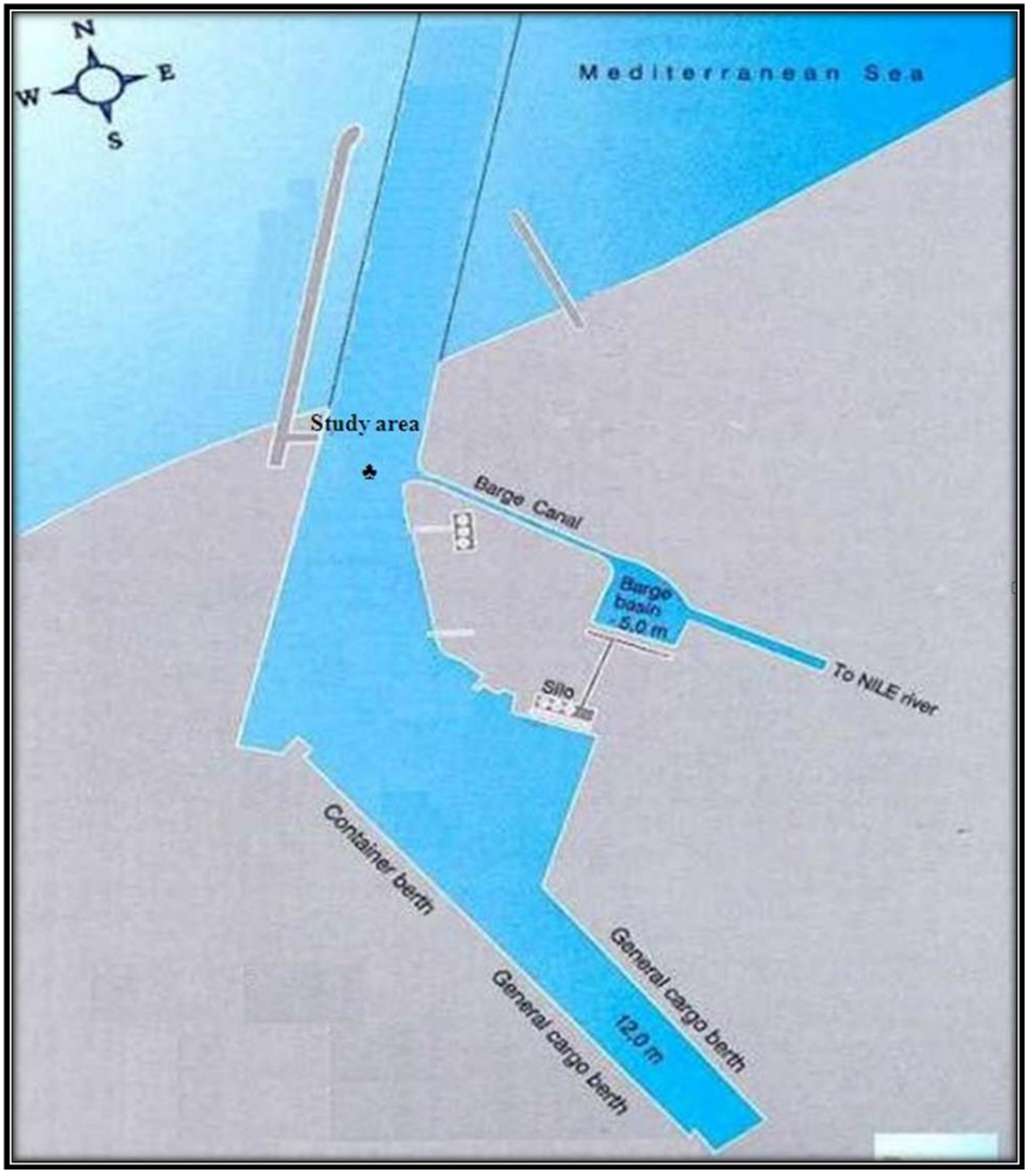
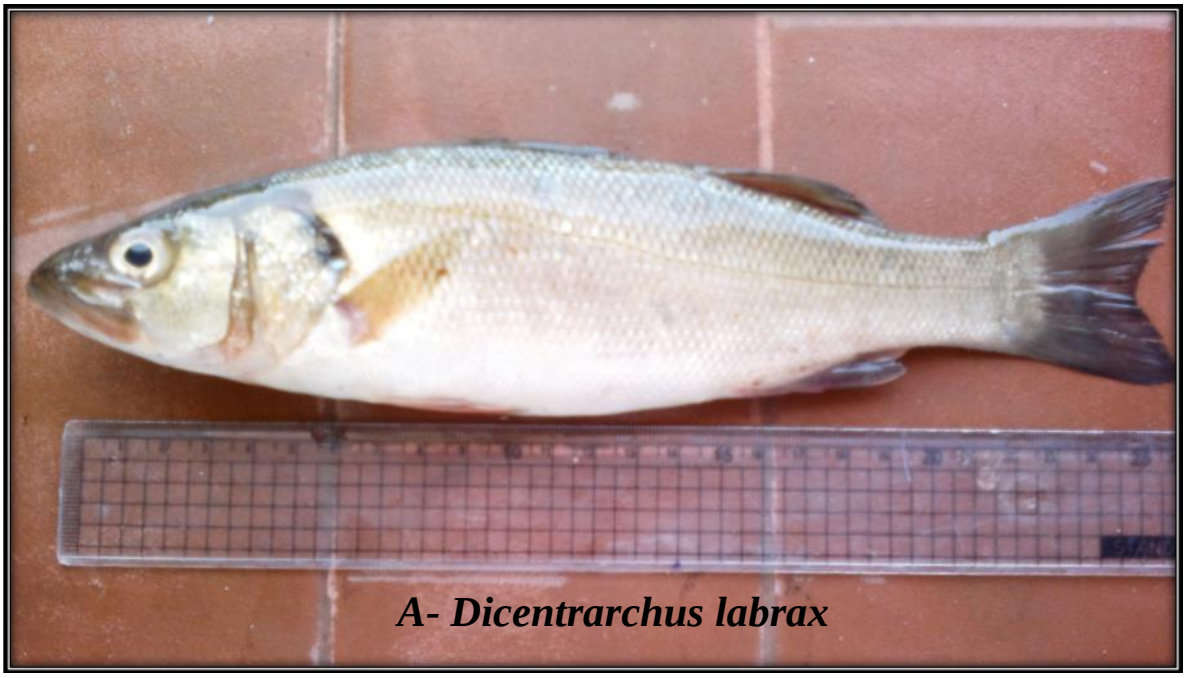
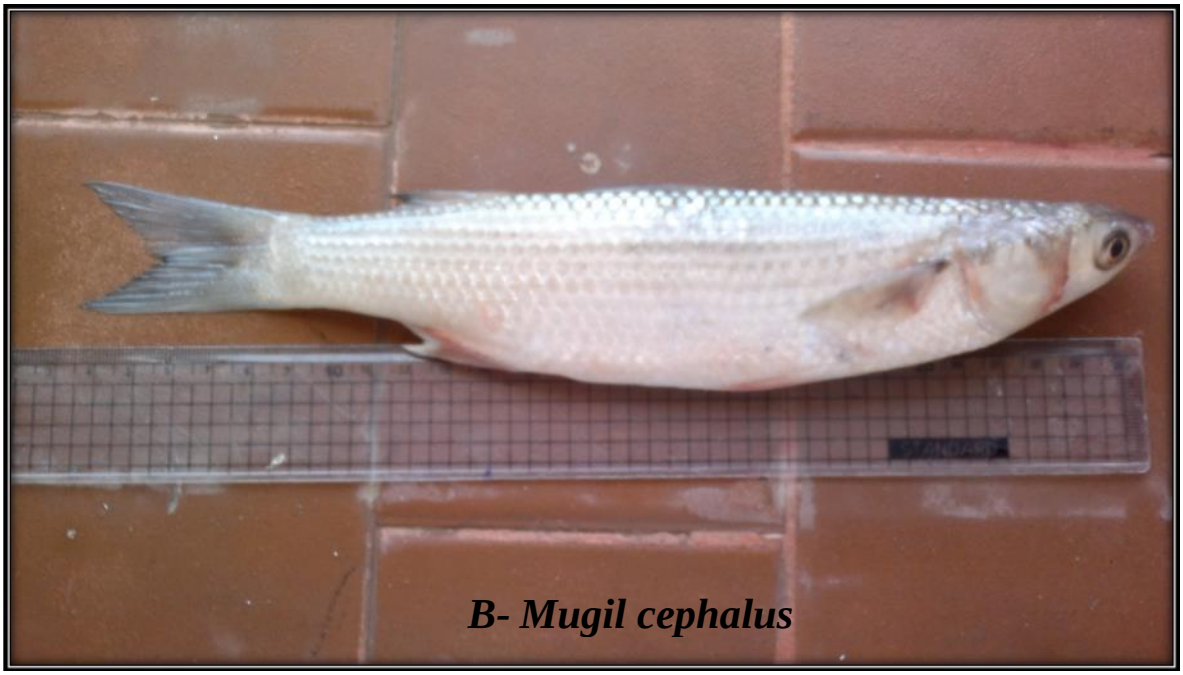


Figure 4: Map showing the study area and fish collection at Damietta port of Egypt.



A- Dicentrarchus labrax



B- Mugil cephalus

Figure (5): Photomicrographs showing the fish species examined during the present study.

The parasitic copepods were identified using **Yamaguti (1954); von Nordmann (1832)** and **Wilson (1911)**. The copepod parasites under investigation are *Lernanthropus kroyeri* **van Beneden, 1851** that collected from *Dicentrarchus labrax* and *Ergasilus versicolor* **Wilson, 1911** that collected from *Mugil cephalus*.

4- Morphological studies of copepods:

A-Light microscopy studies:

Specimens of parasitic copepods from the studied area were examined alive. Each parasite was transferred with a drop of freshly filtered marine water onto a clean slide and covered with a cover slip then one or two drops of formaldehyde were added to fix the parasitic copepod on extension. The specimens were flattened by withdrawn some water and formaldehyde from the space under cover slip with a filter paper until the internal organs were visible.

The collected copepods were fixed in 10% buffered formalin solution for whole mount preparations. The fixative solution is:

10% formalin

Formaldehyde	10ml
Distilled water	90 ml

Carmine stain is the most commonly used for the whole mount preparations. The clarity of a good whole mount results from the limitation of the carmine to the nuclei with the cytoplasmic areas completely or relatively free of the dye. When a whole mount fails to show sharp details, this usually indicates that the stain is too diffuse (over-stain), dehydrated in ascending series of alcohol (80%-100%), cleared in xylene and mounted in DPX. The carmine staining fluid is prepared by the following methods.

Grenacher's Borax Carmine and **Aceto-carmine (Cited from Weesner, 1968).**

Add 4 gm of Borax and 2 gm of Carmine to 100 cc. of distilled water and mix this mixture very well, allow to stand for several days with stirring daily. The solution may be boiled gently and mixed with 100 cc. of 70% alcohol, allow to stand for several days and filter. Add a crystal of thymol to avoid growing of bacteria.

B- Electron microscopy studies:

i-Techniques for scanning electron microscopy:

Some of the specimens cleaned in bi-distilled water then fixed in 2.5% glutaraldehyde to prepared for scanning electron microscopy (SEM). Each parasitic copepod was transferred with a drop of bi-distilled water onto a clean slide and covered with a coverslip then one or two drops of 2.5% glutaraldehyde were added to fix the parasitic copepod on extension. The specimens were well flattened by pressure resulting from withdrawing some water and glutaraldehyde from the space between the coverslip and the slide with a filter paper.

The specimens were fixed in glutaraldehyde fixative at 4ºC for 2-24 hours before washing in 0.1M Sodium cacodylate – Hcl buffer (pH 7.2) for 30 minutes (repeated thrice) and post-fixed in 1% aqueous osmium tetroxide for 1-2 hours **(McDowell and Trump, 1976).** The copepods were further washed in distilled water for 10 minutes (repeated twice) before dehydrated in a series of ethanol (50%, 75%,85%,95% and 100%). The specimens were dehydrated in 1-2 ml of hexamethydisilazane (HMDS) for 10 minutes and air dried at room temperature. The dried sample was mounted onto a SEM specimen stub using a double-sided sticky tape and the sample was coated with gold before viewing under JEOL – JSM 5200 LV Field Emission SEM equipped.

ii-Techniques for transmission electron microscopy:

For semi-thin sections, the parasites were washed using distilled water, then fixed in 2.5% glutaraldehyde buffered to pH 7.3 with 0.1M sodium cacodylate-HCl buffer at 4°C for about 2h. The specimens were washed for at least 1h in several changes of cold buffer (0.1M sodium cacodylate-HCl containing 3% sucrose and 0.1M CaCI2). Post-fixation was carried out using 1% osmium tetroxide in sodium cacodylate buffer at 4°C for 15 minutes to 1h, depending on the freshness of the fixative and the thickness of the specimens.

The specimens were left in washing buffer overnight and were then dehydrated using an ascending series of ethanol solutions. They were placed in three changes of propylene oxide for 10 minutes in each. They were then placed in a mixture of propylene oxide and Araldit resin for 30 minutes, orientated and embedded in capsules containing pure Araldite mixture.

The capsules were placed in an oven overnight at 60°C for 3 days. Semi-thin sections were cut using glass knives at a thickness of 1µm using an LKB ultra microtome and were stained in a 1% solution of toluidine blue in 1% borax. Sections were mounted in DPX and examined using bright field and phase-contrast microscopy.

Ultra-thin sections were cut from the resin blocks at a thickness of 70 nm using glass or diamond knives. The sections were mounted on 75-mesh coated grids (1% parlodion in amyl acetate) and stained in a solution of aqueous or alcoholic uranyl acetate for about 30 minutes followed by lead citrate for about 5 minutes. After drying, they were examined using a JEOL 2000EX TEM operating at 80kV.

5- Histopathological studies:

A-Preparations for light microscopy studies:

Specimens of parasitized and unparasitized gills were fixed in 10% formaldehyde solution for histopathological examination. The specimens were washed under running tap water over night to remove the excess of fixative solution. They were dehydrated in ascending series of alcohol, processed through xylene-alcohol and then cleared in two changes of xylene, 30 minutes each. They were transferred into a mixture of xylene and melted paraffin wax for 1 hour and then into two changes of pure paraffin wax, 30 minutes for each. The specimens were embedded in pure paraffin wax. Serial sections were cut at a thickness of 5-6 µ using rotary microtome. Sections were stained in haematoxylin and eosin (HxE) according to **Drury and Wallington (1967).** Finally, the stained sections were cleared in xylene, mounted in canada balsam and examined under light microscopy.

B-Preparations for electron microscope studies:

Specimens processing of SEM and TEM techniques like written in morphology part.

RESULTS AND DISCUSSIONS

Part 1

Morphological and Anatomical studies

Light Microscopy Studies

Lernanthropus kroyeri **van Beneden, 1851**

- **Systematic position:**

Phylum: Arthropoda

Subphylum: Crustacea

Class: Maxillopoda

Subclass: Copepoda

Order: Siphonostomatoida **Burmeister, 1835**

Family: Lernanthropidae **Olsson, 1869**

Genus: *Lernanthropus* **de Blainville, 1822**

Species: *Lernanthropus kroyeri* **van Beneden, 1851**

Redescription:

The redescription of the copepodian parasite *Lernanthropus kroyeri* is based on the study of thirty living adult parasite individuals and ten mounted specimens collected from the gill filaments of the sea bass fish, *Dicentrarchus labrax.*

(a) Adult female

Body of female *L. kroyeri* is elongated with long egg strings (Figs. 6, 7, 8, 9 & 10). The cephalthorax is narrower anteriorly, posterior margin slightly concave, posterolateral corners rounded, anterolateral extended ventrally as prominent, rounded lobes and curved ventrally on each side.

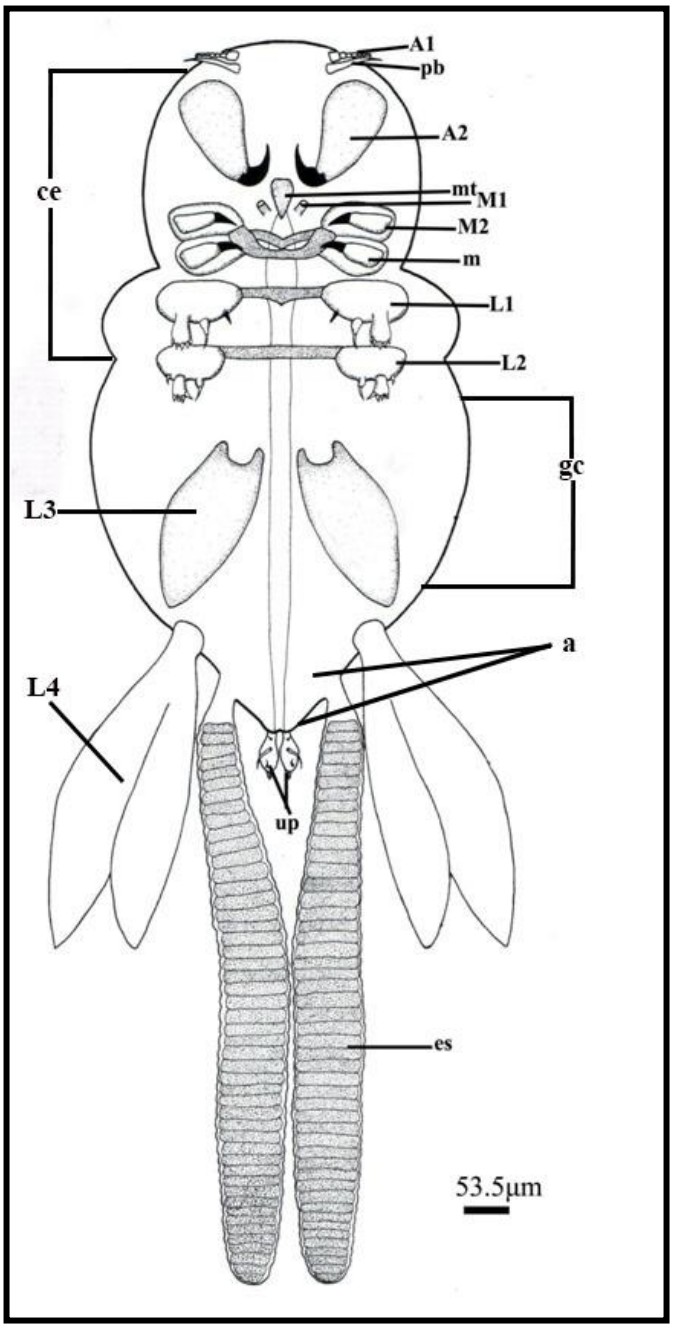


Figure (6):Schematic drawing of ventral view of the female copepodian parasite**,** *Lernanthropus kroyeri* **van Beneden, 1851,** A1, 1st antenna; A2, 2nd antenna; a, abdomen; ce, cephalothorax; es, egg sac; gc, genital complex; L1, 1st thoracic leg; L2, 2nd thoracic leg; L3, 3rd thoracic leg; L4, 4th thoracic leg; m, maxilliped; M1, 1st maxilla; M2, 2nd maxilla; mt, mouth tube; pb, parabasal flagellum and up, uropods.

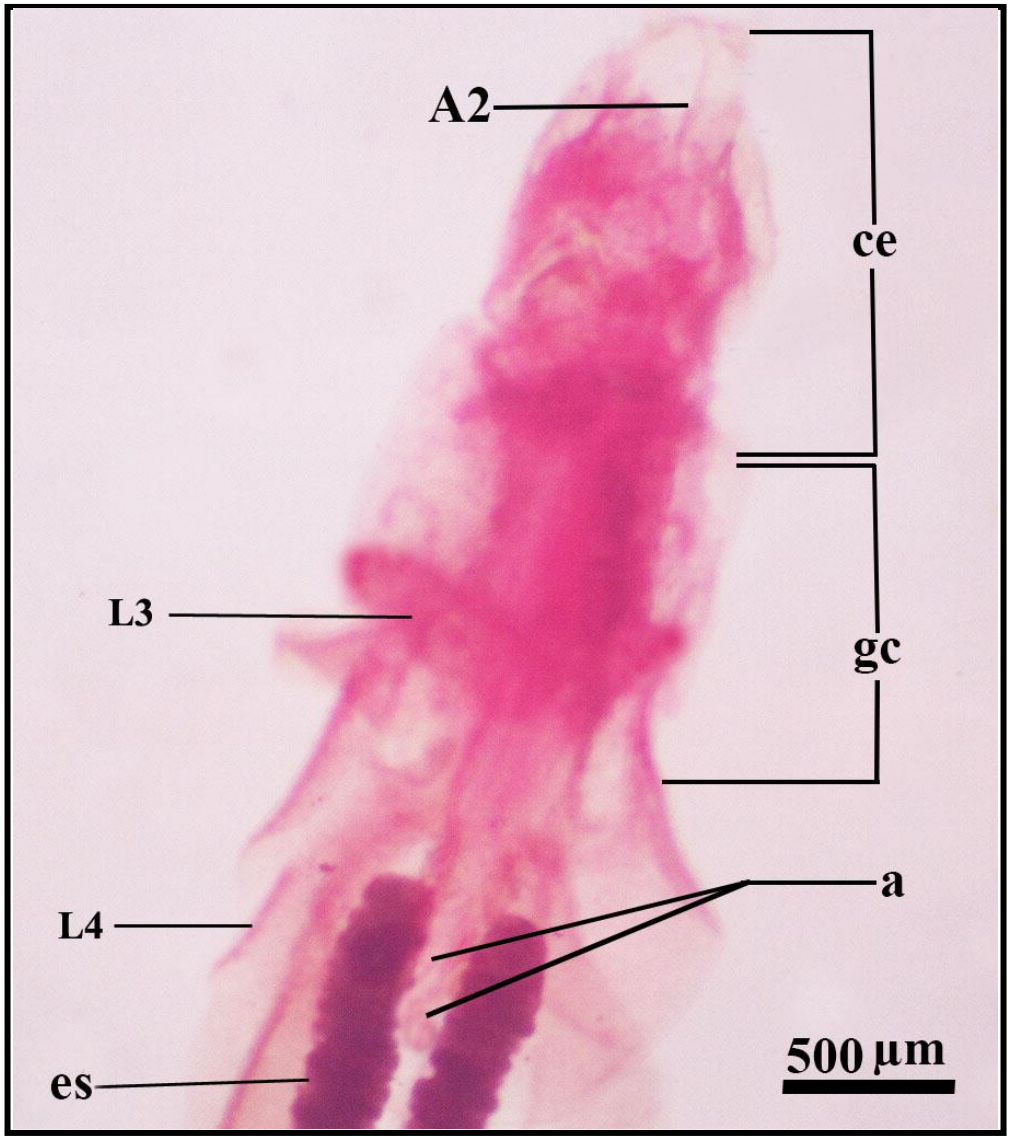


Figure (7): Light microscope photograph showing adult female *Lernanthropus kroyeri*, A2, 2nd antenna; a, abdomen ce, cephalothorax; es, egg sac; gc, genital segment; L3, 3rd thoracic leg and L4, 4th thoracic leg.

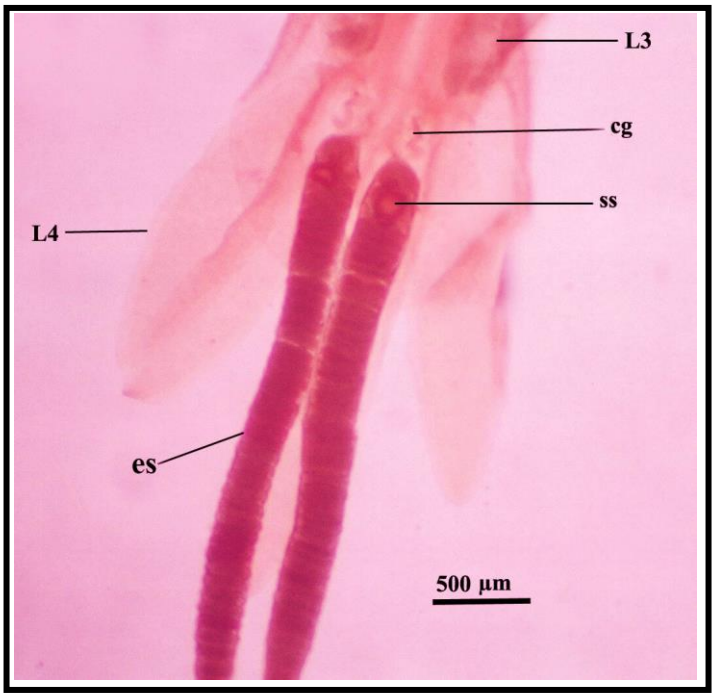


Figure (8): Light microscope photograph showing posterior end of adult female *Lernanthropus kroyeri*, cg, cement glands; es, egg sac; L3, 3rd thoracic leg; L4, 4th thoracic leg and ss, spermatophore sac.

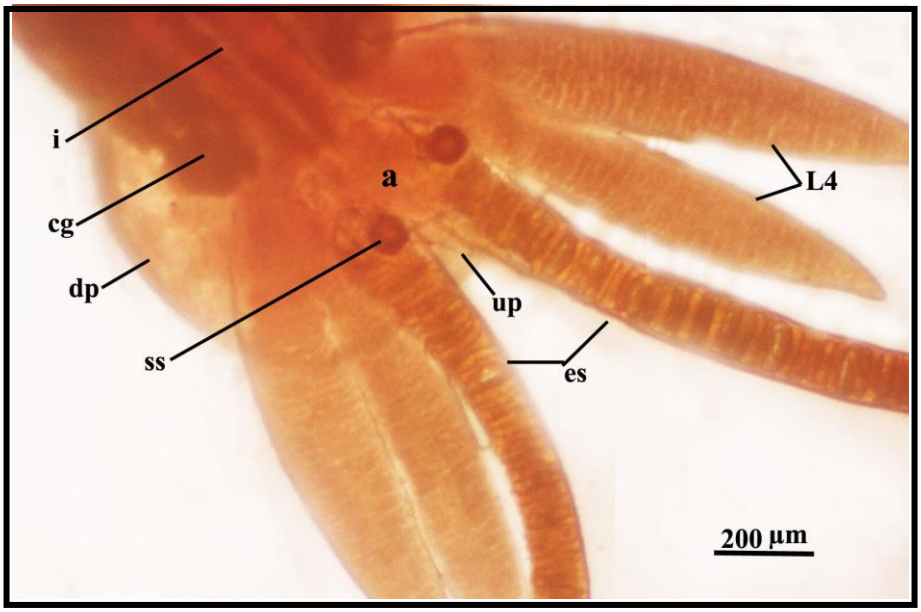


Figure (9): Light microscope photograph showing genital complex of adult female *Lernanthropus kroyeri*, a, abdomen; cg, cement glands; dp, dorsal plate; es, egg sacs; i, intestine; L4, 4th thoracic leg; ss, spermatophore sac; and up, uropod.

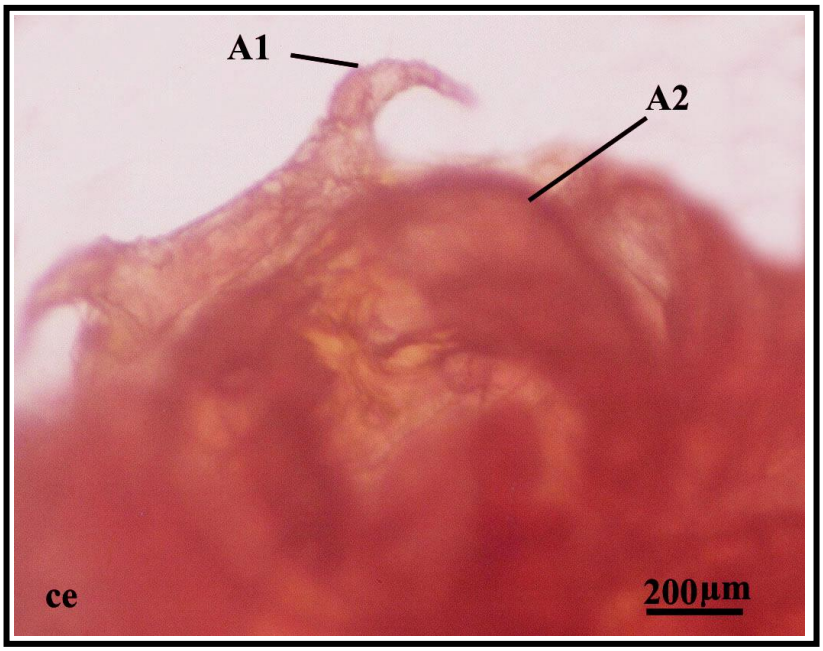


Figure (10): Light microscope photograph showing "enlarged" cephalothorax of adult female *Lernanthropus kroyeri*, A1, first antenna; A2, second antenna and ce, cephalothorax.

The cephalon and first thoracic segment (Fig. 7) fused to form cephalothorax, slightly wider than long. The remaining thoracic segments fused forming genital complex. Genital complex is wider than long. There is deep constriction between cephalothorax and pregenital trunk.

The later is with prominent, rounded anterolateral corners and slightly convex lateral margins. Inside the genital complex there are two oval dorsal ovaries and large ventral cement glands. Inside the abdomen of some females, two spermatophores sacs attach at each vaginal opening (Figs. 8 & 9).

Dorsal shield of genital complex in female expands posteriorly and dorsally, forming a sac (=dorsal plate) with a supporting dorsal layer completely covering abdomen (Fig. 9). Each sac emerged from a genital orifice containing (66-80) disc-shaped eggs (Figs. 8 & 9). Abdomen is short and distinguished at beginning of dorsal plate extension (Fig. 9).

First antenna (Fig. 10) is seven-segmented while the second antenna is two-segmented ended with strong claw for attachment of parasite onto the host tissue (Figs. 7 & 10).

Third thoracic leg (Figs. 7 & 8) is unarmed and long, protruding posteroventrally from medial region of genital complex, parallel to each other. Fourth thoracic leg is bilobed and unarmed, protruding ventrolaterally from distal region of genital complex (Fig. 9).

(b) Adult male

Body of male parasite is elongated (Figs. 11 & 12). Cephalon and first thoracic segment fused to form cephalothorax, slightly wider than long. The head separated by a constriction from the rest of the body. The remaining thoracic segments fused forming genital complex. Genital complex is slightly identical in length and width. Uropod is fusiform and unarmed. Genital complex indistinguishably fused to trunk anteriorly and to abdomen posteriorly.

Abdomen (Figs. 12 & 13) is short could not be clearly delimited in male. There are two spermatophores in posterior vasa deferentia inside the abdomen of males (Fig. 13).

First antenna (Fig. 12) is seven-segmented. Second antenna (Fig. 12) is sturdy and two-segmented.

Maxilliped (Fig. 12) is subchelate, corpus stout unarmed and claw apicaly directed with longitudinal ridges. Uropod (Fig. 12) is unsegmented and fusiform.

Fourth thoracic leg (Fig. 13) is bilobed and unarmed, protruding ventrolaterally from distal region of genital complex.

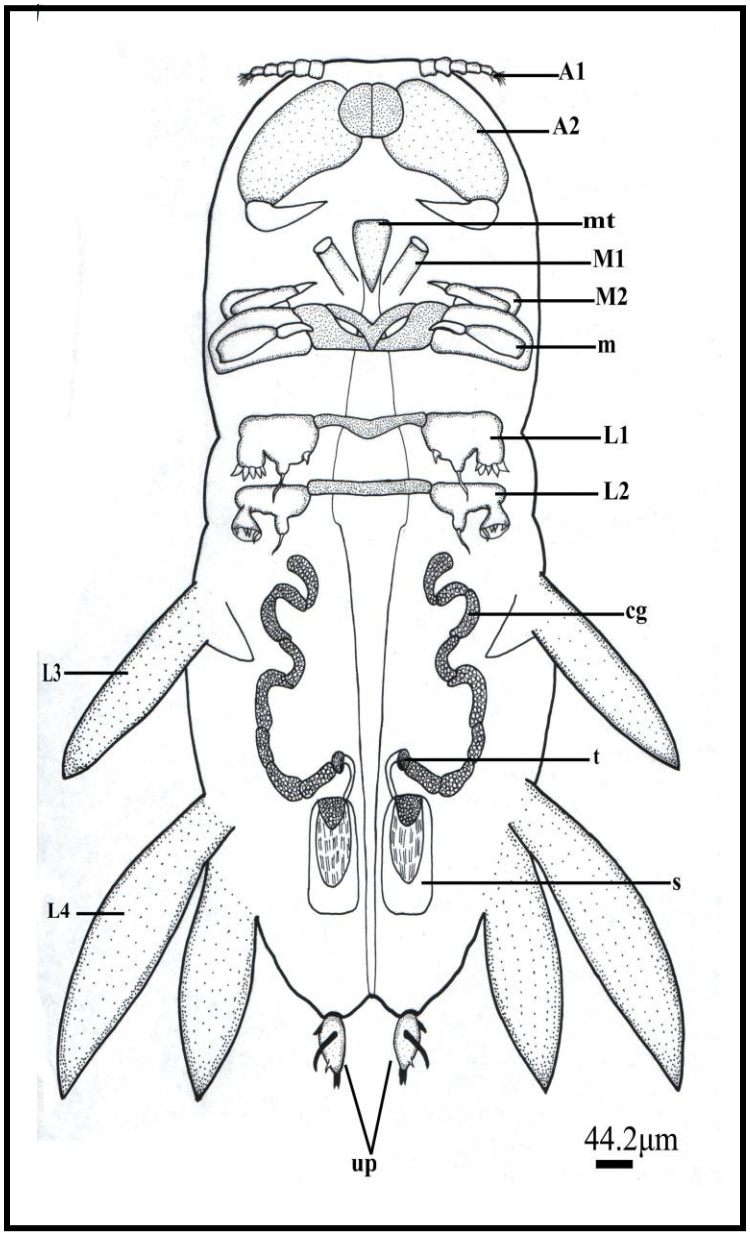


Figure (12):Schematic drawing of ventral view of the male copepodian parasite**,** *Lernanthropus kroyeri* **van Beneden, 1851,** A1, 1st antenna; A2, 2nd antenna; cg, cement glands; es, egg sac; gc, genital complex; L1, 1st thoracic leg; L2, 2nd thoracic leg; L3, 3rd thoracic leg; L4, 4th thoracic leg; m, maxilliped; M1, 1st maxilla; M2, 2nd maxilla; mt, mouth tube; pb, parabasal flagellum and up, uropods.

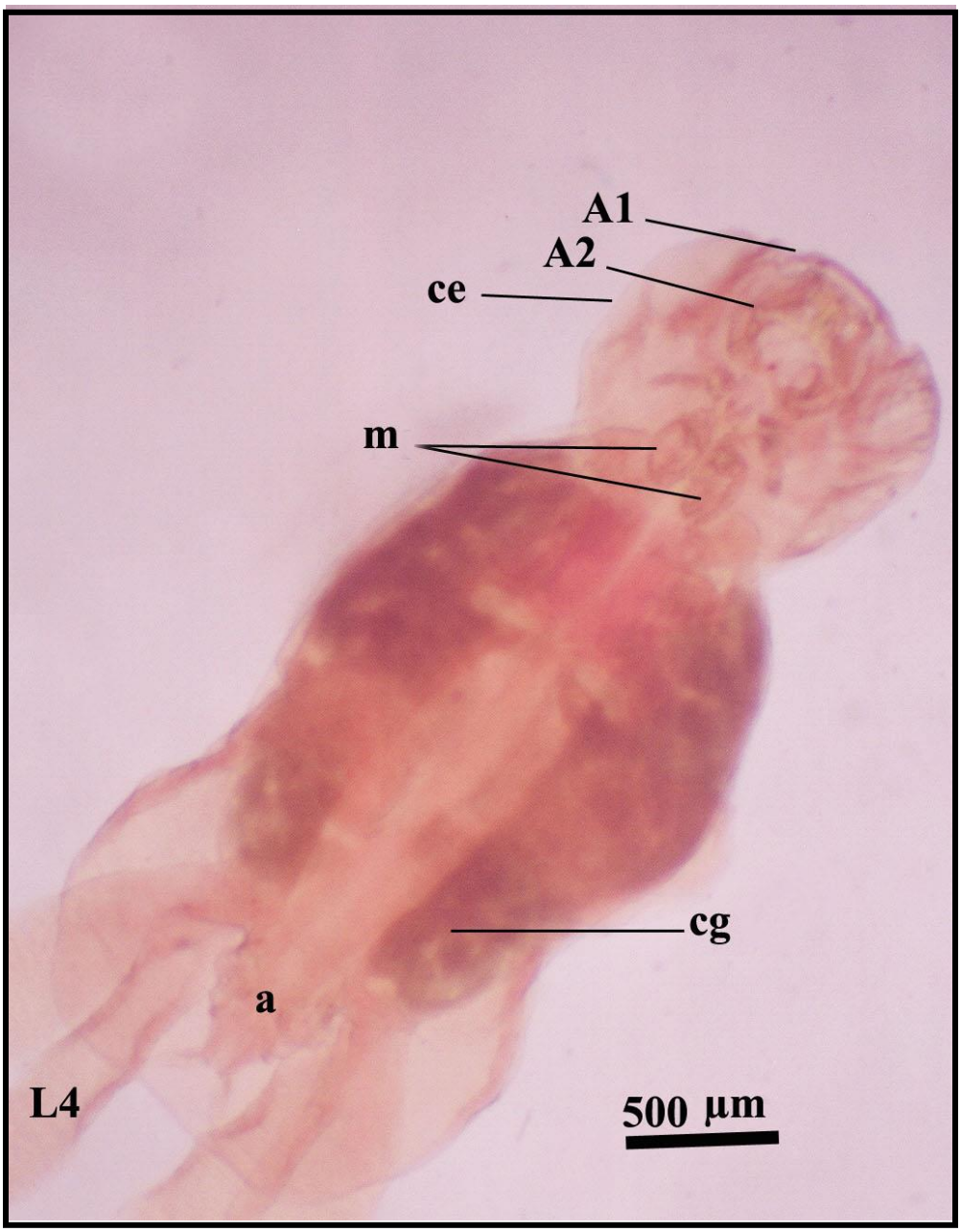


Figure (12): Light microscope photograph showing adult male *Lernanthropus kroyeri*, A1, 1st antenna; A2, 2nd antenna; ce, cephalothorax; m, maxilliped; cg, cement gland; L4, 4th thoracic leg and a, abdomen.

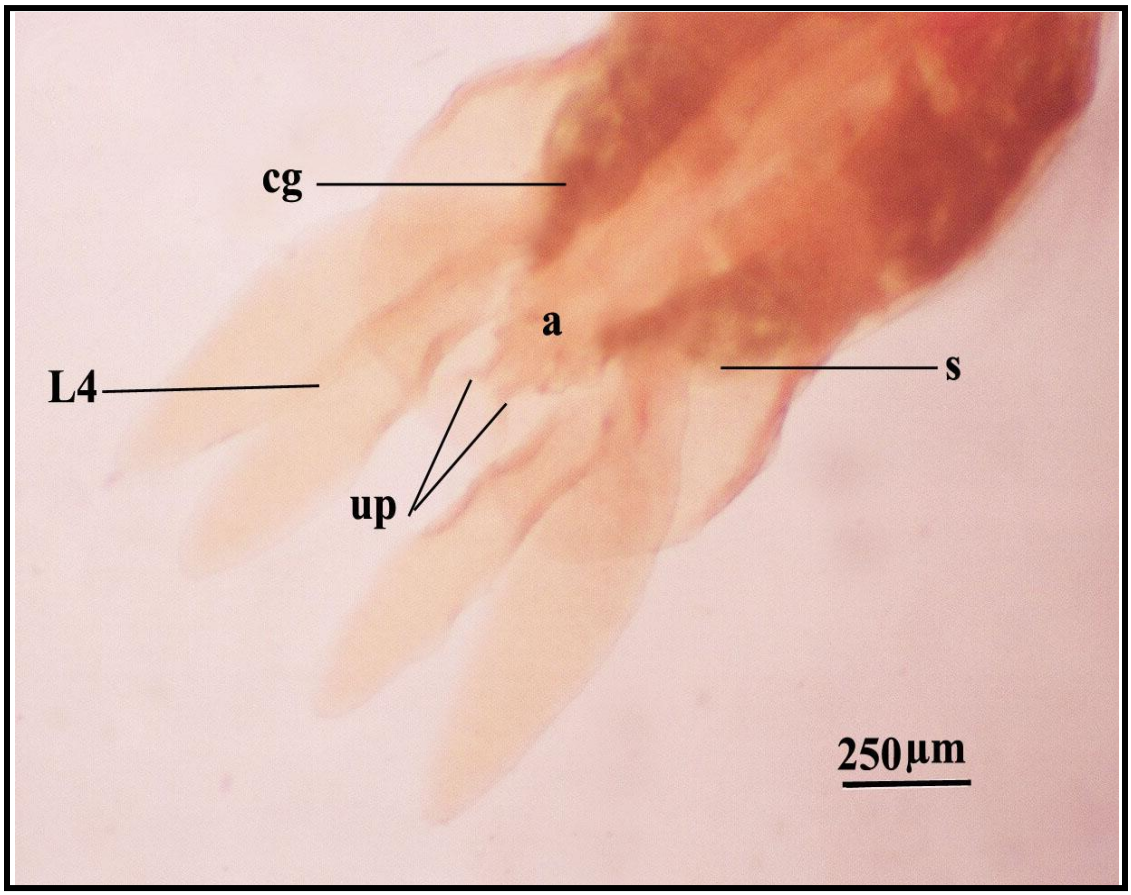


Figure (13): Light microscope photograph showing adult male *Lernanthropus kroyeri*, cg, cement gland; s, spermatophore; L4, 4th thoracic leg; up, uropods and a, abdomen.

Discussion:-

Crustacean parasites are numerous and have a worldwide distribution, in fresh, brackish and salt waters **Kabata (1970)**. Copepods comprise the largest group of crustacean parasites on fish, numbering more than 1000 species **Kabata (1970, 1979a)**. Data on the structural and functional properties of host tissue, as well as the anchorage modality and feeding habits of parasitic crustacean copepods, are available **Kabata (1970)** and **Pike and Wadworth (2000).**

The Lernanthropidae is the 3rd-largest family of fish-parasitizing Siphonostomatoida beside Lernaeopodidae and Caligidae. The family contains over 150 species, with a great majority of them occurring in tropical waters. According to the copepod database produced by **Boxshall (2011)** *Lernanthropus* is the largest genus of lernanthropids comprising 111 species. In this study, the parasitic copepod *Lernanthropus kroyeri* **Beneden, 1851** infesting gills of sea bass fish, *Dicentrarchus labrax*.

The anatomical structure demonstrated in the present description are evidently to suggest that the present species described here in belongs to genus *Lernanthropus* **de Blainville, 1822** according to the following generic criteria which based by **Yamaguti and Yamasu (1960), Hewitt (1968**) and **Kabata (1971**). These generic morphological criteria are:

The cephalothorax of the female is fused with first leg-bearing segment, dorsally with well developed shield curved ventrally on each side. Abdomen is small, indistinctly one or two segmented. Egg sacs are straight or irregularly coiled under plate of fourth leg-bearing segment and uniseriate. First antenna is uniramous, usually indistinctly segmented, with or without parabasal flagellum. Second antenna is subchelate. Maxilliped is subchelate. First leg is small, biramous and rami one-segmented. Second leg is similar to first. Third is modified

into variously shaped, plate-like structure and with or without foliaceous outgrowths of sympod and exopod. Fourth leg is unsegmented, bilobed, large or small and with or without filiform tips. Fifth leg is uniramous, small or absent. Uropods are present. The cephalothorax of the male is with dorsal shield flat. Abdomen is one-segmented. First antenna is uniramous and usually indistinctly segmented. Second antenna is subchelate. Maxilliped is subchelate. First leg is small, biramous and rami one-segmented. Second leg is similar to first. Third and fourth legs are uniramous or bilobed, lobes are flat and comparatively long. Uropods are present.

The present individuals of the parasitic copepod *Lernanthropus kroyeri* **Beneden, 1851** infesting gills of sea bass fish, *Dicentrarchus labrax* were collected previously, from the sea bass fish, *Dicentrarchus labrax* in Egyptian Mediterranean coast by **Abu Samak (2004**). *Lernanthropus kroyeri* has been recorded from many localities along the coast of Europe, the Irish Sea and the coast of Norfolk on the sea bass fish, *Dicentrarchus labrax* **(Wilson, 1922)**.

Lernanthropus kroyeri has been recorded from many localities along the coast of Europe, from the Adriatic to the southern North Sea appears to be *Dicentrarchus labrax* **(Kabata, 1979b)**. An unusual record *Lernanthropus kroyeri* was identified as being parasitic on *Lutianus griseus* from the Gulg of Mexico **(Bere, 1936)**. Most of the investigations about *Lernanthropus kroyeri* of the sea bass fish, *Dicentrarchus labrax* in Turkey are focused on the Aegean Coast **(Tokşen, 1999)**. From the eastern Mediterranean coast, *Lernanthropus kroyeri* **Beneden, 1851** was recorded for the first time on gills of the sea bass fish, *Dicentrarchus labrax* by **Abu Samak, 2004**.

The present redescription of the parasitic copepod *Lernanthropus kroyeri* **Beneden, 1851** infesting gills of sea bass fish, *Dicentrarchus labrax* is similar to

the description of **Abu Samak (2004)**. **Kabata and Gusev (1966)** found that some species might differ considerably in size, and they suggested that the size differs in the same parasite, depending on the geographic area. The armature setation number on the segments of female and male first antennas in the present study differs from that of *Lernanthropus gisleri* which is similar to *L. kroyeri* in the study by **Kabata (1979b)** and resembles that of the young female and male *L. kroyeri* in the study by **Cabral *et al*. (1984)**.

Ergasilus versicolor **Wilson, 1911**

- **Systematic position:**

Phylum: Arthropoda

Subphylum: Crustacea

Class: Maxillopoda

Subclass: Copepoda

Order: Poecilostomatoidea **Thorell, 1859**

Family: Ergasilidae **von Nordmann, 1832**

Genus: *Ergasilus* **von Nordmann, 1832**

Species: *Ergasilus versicolor* **Wilson, 1911**

Redescription:

The redescription of the copepodian parasite *Ergsilus versicolor* is illustrated in (Figs. 14, 15, 16, 17 & 18).

The following description is based on the study of fifteen living adult parasite individuals and seven mounted specimens of *Ergasilus versicolor* collected from the gill filaments of the Flathead Grey Mullet, *Mugil cephalus*.

The body consists of two main parts, prosome and urosome. The prosome consists of cephalosome and mesosome. The first somite of the mesosome is fully incorporated into the cephalosome forming cephalothorax which is equal approximately in length to the remaining part of the body. There is no boundary between them is indicated only by a shallow, indistinct constriction, just posterior

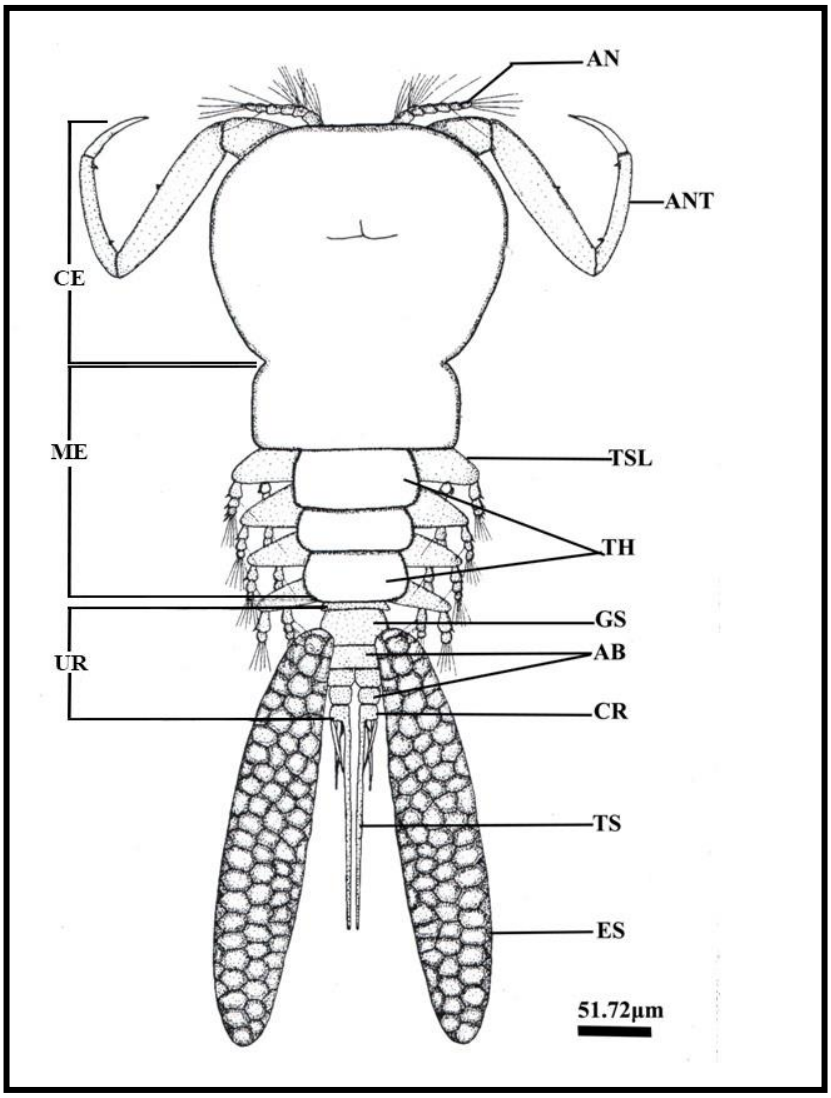


Figure (14):Schematic drawing of dorsal view of the female copepodian parasite**,** *Ergasilus versicolor***,** AB, Abdomen; AN, 1st antenna; ANT, 2nd antenna; CE, Cephalosome; CR, Caudal ramus; ES, Egg sac; GS, Genital segment; ME, Mesosome; TH, Thorax; TS, Terminal seta; TSL, Thoracic swimming leg and UR, Urosome.

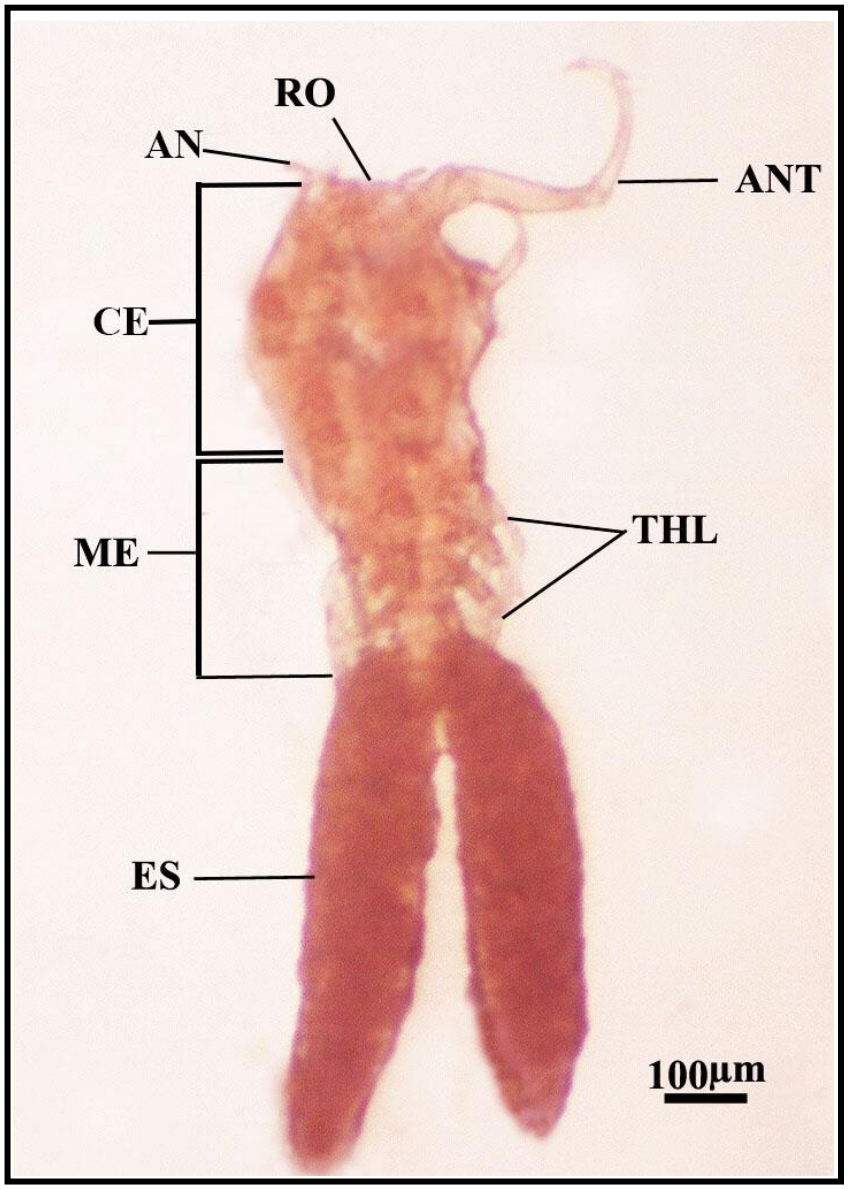


Figure (15): Light microscope photograph showing adult female *Ergasilus versicolor*, AN, 1st antenna; ANT, 2nd antenna; CE, cephalosome; ES, egg sac; ME, mesosome; RO, rostrum and THL, thoracic swimming legs.

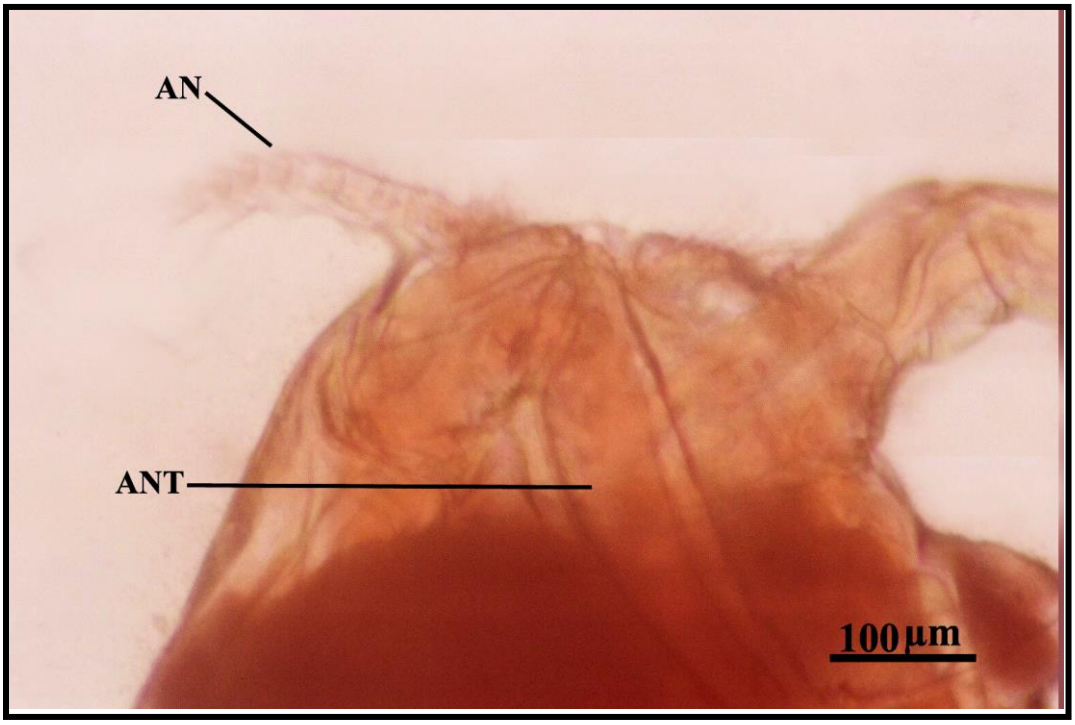


Figure (16): Light microscope photograph showing 1st antenna (AN) of adult female *Ergasilus versicolor*; ANT, 2nd antenna.

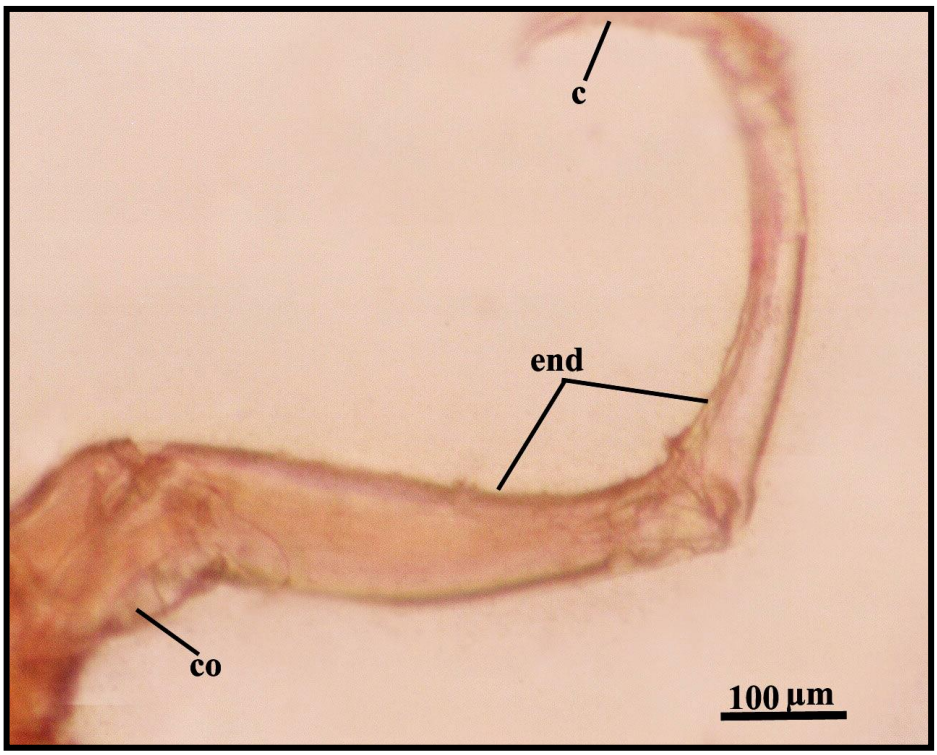


Figure (17): Light microscope photograph showing the structure of 2nd antenna of adult female *Ergasilus versicolor*; c, claw; co, coxobasis and end, endopodal segments.

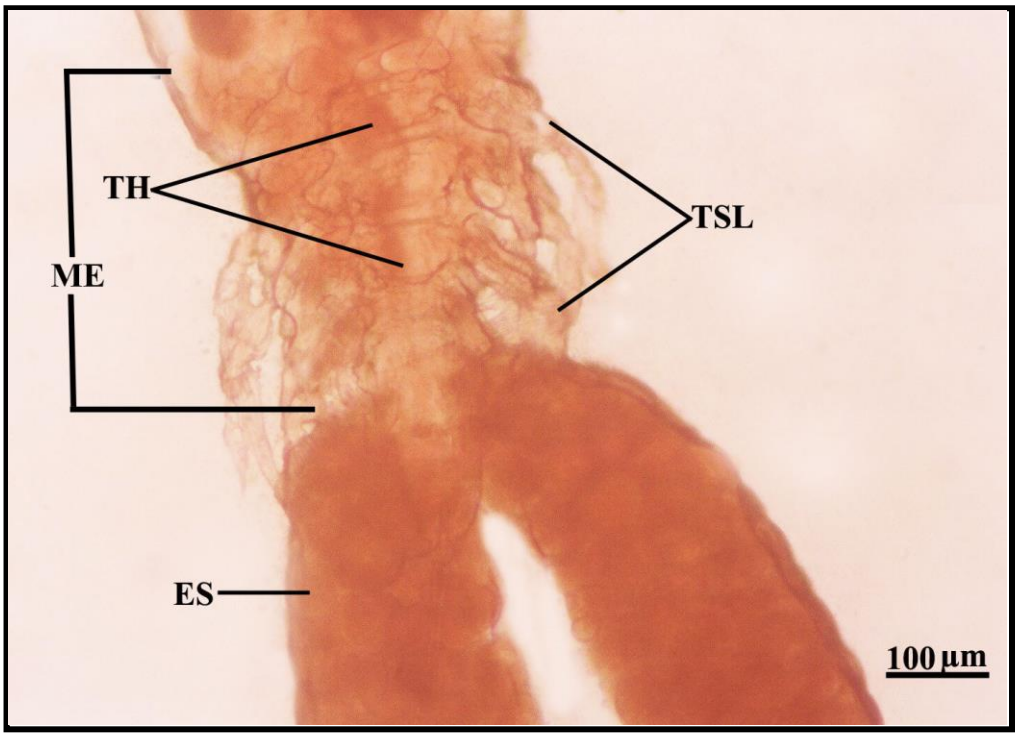


Figure (18): Light microscope photograph showing mesosome of adult female *Ergasilus versicolor*, ES, egg sac; ME, mesosome; TH, thorax and TSL, Thoracic swimming legs.

to the anterior two thirds, and running across both lateral sides (Fig. 15). The cephalothorax (Figs. 14 & 15) is oblong, uninflated and bullet-shaped. Its anterior end is slightly tapering with slightly projecting antennary region forming a short rostrum and the posterior margin is transversely truncated. The cephalothorax decreases in width posteriorly.

The mesosome (Figs. 14 & 15) consists of three free somites comprising second, third and fourth thoracic somites. The metasomal somites are broader than long, progressively narrowed from cephalothorax and decrease in size towards the posterior end of the body.

There are a pair of elongated, maggot-shaped, multiseriated egg sacs (Fig. 15) are originated one from each ventro-lateral side of the genital somite. The egg sacs are long and constituting more than half the total body length. Each egg sac extends posteriorly with slightly tapering and rounded distal end. They are filled with a large number of eggs. Eggs are large, spherical and visible through the thin membrane of the eggs.

Cephalthorax bears one pair of antennules, one pair of antennae. The pair of short cylindrical, setiferous, and segmental antennules are located on the protuberant rostral area. Each antennule consists of six segments (Fig. 16).

A pair of stout prehensile, subchelate and segmented antennae (Fig. 17) is situated on the slightly protruding rostral area. They are long and slender. Each antenna consists of four segments. The first or basal segment is the coxobasis. It is short, slightly cubical in shape and bears a single tooth-like spine arising near its inner distal margin. The second segment is the first endopodal segment which is the longest one. It is widest at its proximal end and narrows towards its distal end.

The third antennary segment is the second endopodal segment and is called the subchela. It is narrow and arched with the sides almost parallel and clearly bent inwards. The fourth or distal antennary segment is the fourth endopodal segment. It is in the form of a stout pointed, clasper-like smooth claw lacking tooth. It is the shortest segment.

Five pairs of thoracic swimming legs (Fig. 18) originate from the lateral side of the first five thoracic somites. The first pair of thoracic swimming legs originates ventral-laterally from the posterior part of the cephalothorax and the last pair projects from the fifth thoracic somite. The first four pairs are biramous and exist in a completely form. Each leg consists of a proximal coxapod and a distal basipod which bears the two free rami; an exopod and an endopod. Whereas the fifth thoracic pair is greatly reduced.

The urosome consists of the two last thoracic somites (fifth and sixth), the abdominal somites and the caudal rami. The fifth thoracic somite is extremely reduced, very short and narrow but distinctly apart as much from the fourth thoracic segment as from the sixth thoracic segment that follows it. The sixth thoracic somite is the genital somite. It is a large somite, slightly broader than long and, wide in the middle.

Discussion:-

In all cases the ergasilids were attached to the gills, which are the main site of infection **(Roberts 1970, Boxshall and Montú 1997).** The morphological and anatomical structures demonstrated in the present redescription are evidently to suggest that the present species described herein belong to genus *Ergasilus* **von Nordmann**, **1832** according to the following generic criteria which based by **Markewitsch (1956), Gusev (1962), Yamaguti (1963), Hoffman (1967, 1977), Kabata (1988), Mitchum (1995)** and **Hoffman and Williams Jr. (1999**). These generic morphological criteria are: Body is cyclops-like, expanded anteriorly, tapering to posterior end. Head fused with, sometimes separated from, first thoracic segment and more or less fused with second thoracic segment forming cephalothorax which may be highly inflated dorsally, line of fusion usually visible, either throughout length or only at sides of body. An eye spot may be evident on the cephalothorax dorsally, medially and toward the anterior end. Fourth free thoracic segments (fifth thoracic segment) are small, occasionally completely imperceptible. Sixth thoracic segment is bearing oviductal openings. Abdomen is three segmented in female. Caudal rami short, well developed, each provided with four setae.

Egg sac long, often maggot-shaped or rather pump, eggs small and numerous. First antenna (antennule), five or six segmented, setiferous, second antenna subchelate, prehensile, four or five segmented, the terminal segment in the form of a prominent, stout, calsper-like claw.

First pair of swimming legs born on cephalothorax; first four pairs of swimming legs are biramous. Each ramus consists of three segments, except for exopodite of the fourth pair which contains two or occasionally one segment. Fifth pair uniramus, one or two segmented or reduced to a papilla and one, or two setae, rarely obsolete.

Abdelhalim *et al*. (1993), Alston *et al*. (1993) and Alston *et al*. (1996) differentiated between the species of the genus *Ergasilus* **von Nordmann, 1932** on the basis of shape of cephalothorax, presence or absence of dorsal sculpture, ratio of antenna length to length of the cephalothoax, ratio of length of the cephalothorax to the total body length, size ratio of length to width of the cephalothorax, head either fused with or separated from first thoracic segment and more or less fused with second thoracic segment and the way of fusion either partial or complete fusion, number of segments of the antennule and their armature formula, size of the antenna, distal ends joined or unjoined, ratio of length of the last antennary segment to the length of the claw, number and location of spines and sensilla occurring on the segments of the antenna, size ratio of genital segment to fifth thoracic segment, size ratio between abdominal segments, bifurcation of the abdominal segments, ratio of length to width of caudal rami, setation of caudal rami, presene or absene of setae on the dorsal and ventral surfaces of the body segments, setation on the ventral surface of the abdominal segments, ratio of egg sacs (ovisacs) to total body length, size of eggs, armature of swimming legs and structure of the fifth swimming legs.

According to the previous differential criteria, described specimen in the present study is closely related to *Ergasilus versicolor* **Wilson, 1911.**

The present individuals of the parasitic copepod *Ergasilus versicolor* **Wilson, 1911** was recorded for the first time in Egyptian Mediterranean coast infesting gills of the flat-head grey mullet; *Mugil cephalus. Ergasilus versicolor* **Wilson, 1911** was previously collected from the gill filaments of the catfishes, *Leptops ollvaris* of the Mississippi. **Wilson (1911)** described it from the gills of the Fulton cat, *Ictalurus Anguilla & Ictalurus punctatus* in the Mississippi. **Boxshall *et al*., (2002)** collected it from *Aspistor luniscutis* in Brazil and also **Tavares (2005)** described it at the same site, from *Aspistor luniscutis* in Brazil.

Part 1

Morphological and Anatomical studies

Scanning electron microscope Studies

Lernanthropus kroyeri van Beneden, 1851

Fine morphological criteria of *Lernanthropus kroyeri* in the present study are shown in detail.

a) Female:

Body surface ventrally ornamented with patches of setules and elongated with long egg sacs (Fig. 19). Cephalothorax with dorsal shield slightly is narrower anteriorly, anterolateral corners are more rounded than posterolateral corners in dorsal view. Deep constriction between cephalothorax and pregenital trunk. Latter with prominent, rounded anterolateral corners and slightly convex lateral margins (Figs. 20 & 21).

I. Cephalothorax appendages:-

- Maxillule (first maxilla) (Figs. 20,21& 22) is bilobate with inner lobe smaller than outer. Exopod is short, rounded in distal end with two terminal small spines and sub terminal horny spine and setule cover. Endopod sub cylindrical with three apical strong spiniform processes, setule cover.
- Maxilla (second maxilla) (Fig. 20) is uniramous, brachiform and two-segmented: proximal segment (= lacertus) large elongate unarmed; second segment (= brachium) slender with two sub terminal spines (one on anterior margin and other on posterior margin), rows of minute spines and terminal spiny claw armed with two sharp denticles rows, each comprising 13 teeth.

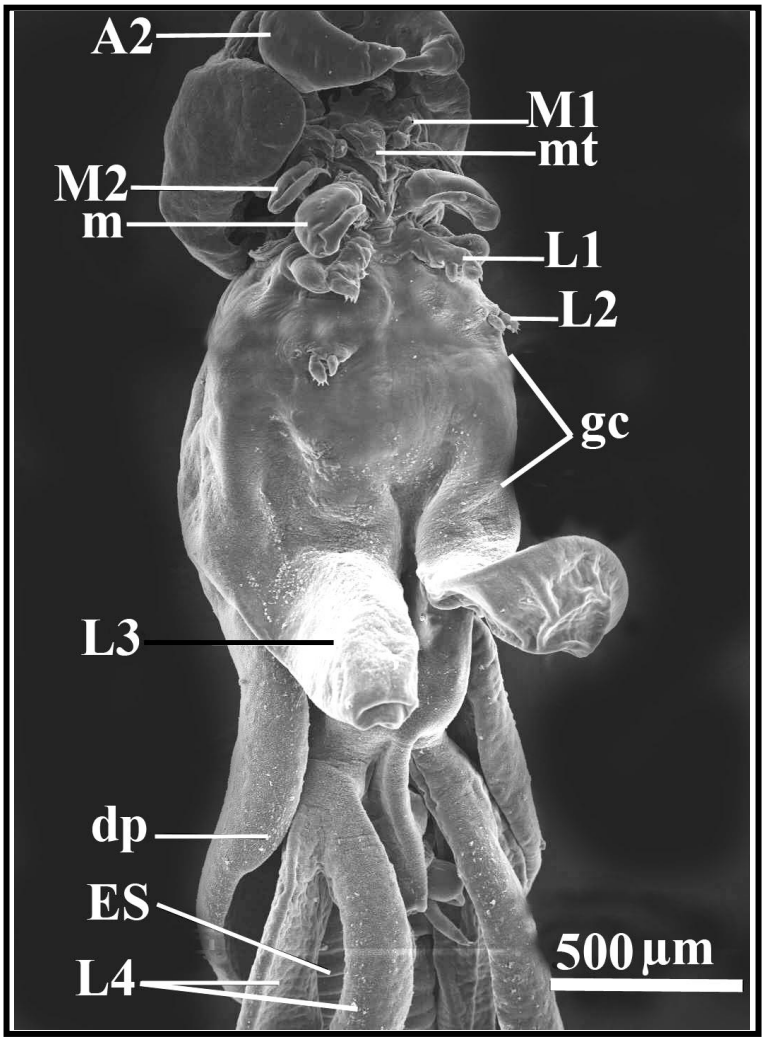


Figure (19): Scanning electron micrograph of adult female *Lernanthropus kroyeri* in ventral view; A2, 2nd antenna; dp, dorsal plate; ES, egg sac; gc, genital complex; L1, 1st thoracic leg; L2, 2nd thoracic leg; L3, 3rd thoracic leg; L4, 4th thoracic leg; M1, 1st maxilla; M2, 2nd maxilla; m, maxilliped; and mt, mouth tube.

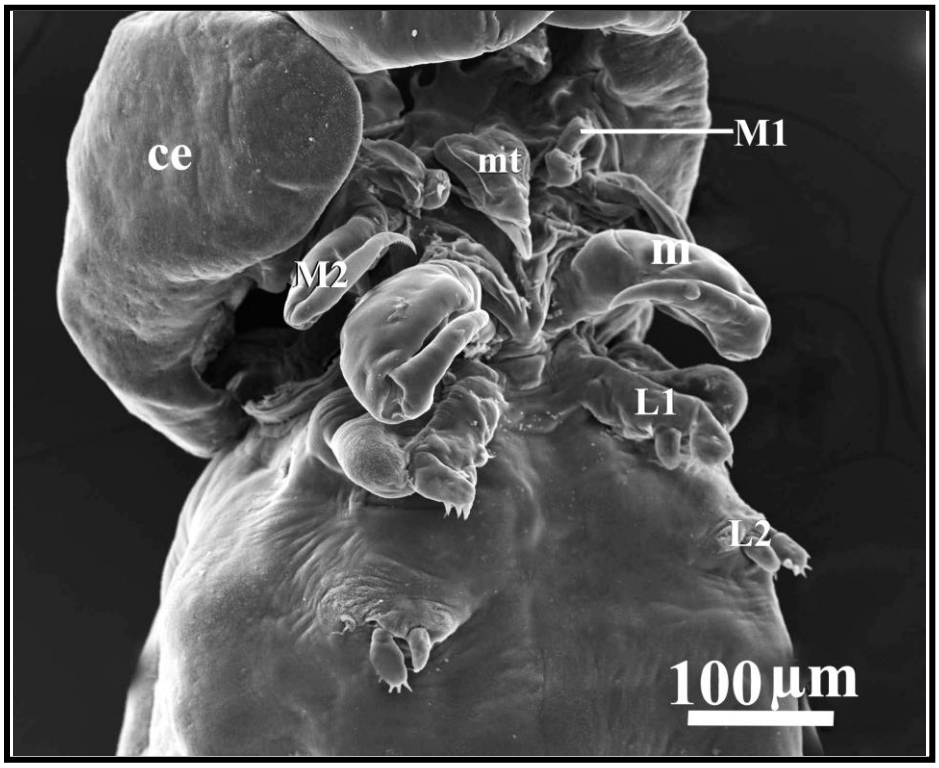


Figure (20): Scanning electron micrograph of adult female *Lernanthropus kroyeri* in ventral view showing cephalothorax appendages, ce, cephalothorax; M1, 1st maxilla; M2, 2nd maxilla; m, maxilliped; mt, mouth tube; L1, 1st thoracic leg and L2, 2nd thoracic leg.

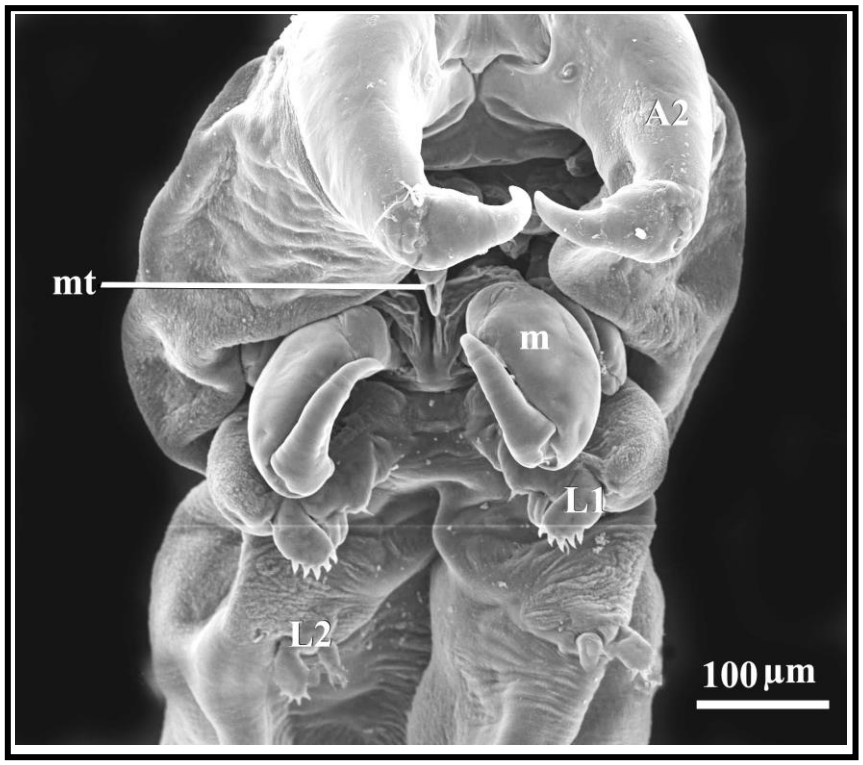


Figure (21): Scanning electron micrograph of adult female *Lernanthropus kroyeri* in ventral view showing cephalothorax appendages, A2, 2nd antenna; m, maxilliped; mt, mouth tube; L1, 1st thoracic leg and L2, 2nd thoracic leg.

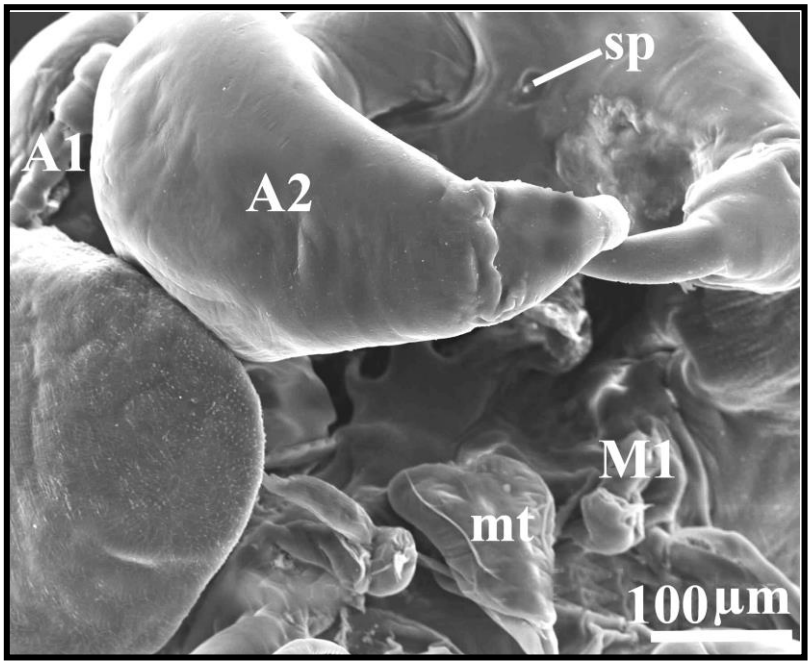


Figure (22): Scanning electron micrograph of adult female *Lernanthropus kroyeri* in ventral view showing (Enlarged) cephalothorax, A1, 1st antenna; A2, 2nd antenna; mt, mouth tube; M1, 1st maxilla and sp, spiniform process.

- Maxilliped is robust and subchelate (Figs. 20 & 21) corpus stout unarmed, subchela armed with single subterminal seta on inner margin and claw apicaly directed with longitudinal ridges.
- Mouth tube (Figs. 20 & 22) is small and sharply pointed distally with tip directed posteriorly, situated between maxillae, the labrum shorter than labium, with some integumental processes and with tube-like buccal stylet. Labium is tapering towards tip with denticulate margins.
- Antennule (first antenna) (Fig. 22) seven-segmented; first segment with one seta on anterior margin, second segment with three setae (two on anterior margin and one on posterior side), third segment with one seta on anterior margin, fourth segment with three setae on anterior margin, fifth segment with one seta on anterior margin, sixth segment with two setae on anterior margin, terminal segment with 8 setae, three apical setae on anterior margin and five subapical setae on posterior side.
- Second antenna is robust and sturdy (Fig. 22) two-segmented; corpus large, curving inwards and tapering distally with single small myxal process. Subchela is curving inwards with a spiniform process (Fig. 22) on inner surface close to the base.

II. Thoracic Legs:-

- First thoracic leg (Figs. 19, 20 & 21) is biramous with large setule covered sympod bearing one slightly curved, stout spine medial to endopod: endopod with spicules distally, bearing one long apical seta: exopod with five prominent spines on terminal margin, inner surface of spines denticulate.

- Second thoracic leg is smaller than first (Figs. 19, 20 & 21) and is biramous: endopod is cylindrical, tapering to short apical seta; exopod with four distal spines, spicules less conspicuous, sympodial process lateral to exopod, with filiform terminal seta.
- Third thoracic leg (Fig. 19) is large, foliaceous and protruding posteroventrally from medial region of genital complex, slightly folded along longitudinal axis.
- Fourth thoracic leg (Figs. 19 & 23) is bifurcate and unarmed, protruding ventrolaterally from distal region of genital complex.

III. Genital complex and abdomen:-

The remaining thoracic segments of parasitic copepod *L. kroyeri* is combined to form the genital complex. The shape of genital complex is broader than long. There is profound constriction between cephalothorax and pregenital trunk (Fig. 19). The last is with distinguished, rounded anterolateral corners and slightly convex lateral margins. There are two spermatophores sacs (Fig. 23) attaches at each vaginal opening inside the abdomen of some females.

The dorsal armor of genital complex in female expands posteriorly and dorsally, forming dorsal plate covering the abdomen (Fig. 19). The abdomen is short, one or two segmented and distinguished at beginning of dorsal plate extension (Fig. 23). Egg strings is usually long trailing behind the body from genital segment and uniseriate with numerous disc-shaped eggs (Fig. 23). Caudal rami(=uropods) is unsegmented, terminal (Fig. 23) and fusiform with 5 setules (two terminal and three subterminal).

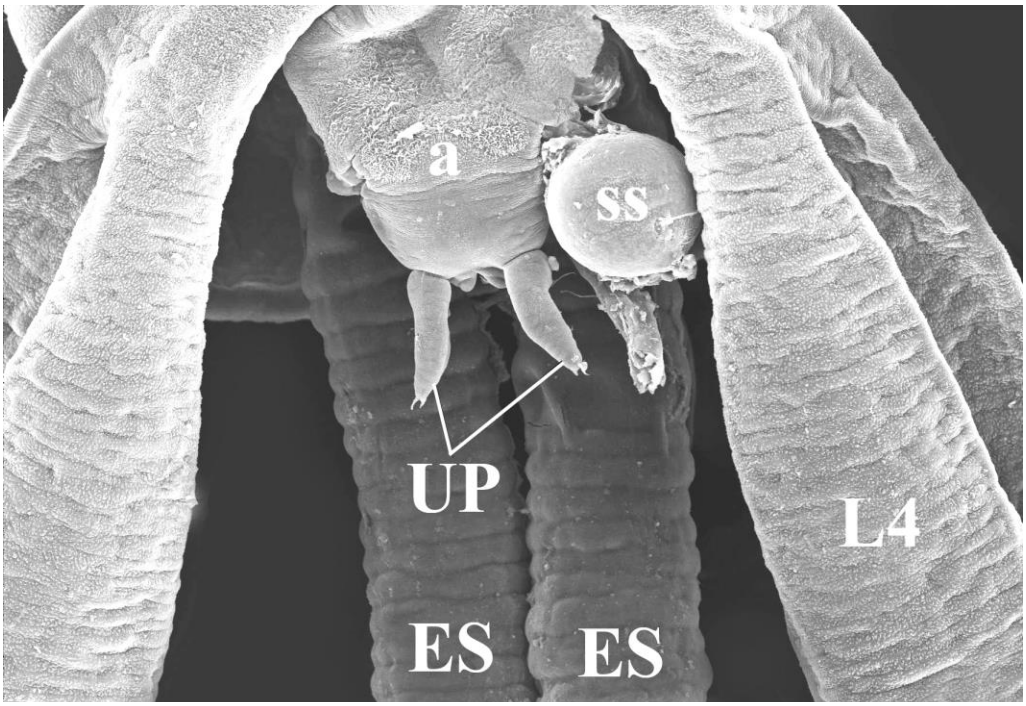


Figure (23): Scanning electron micrograph of adult female *Lernanthropus kroyeri* in ventral view showing the abdomen and egg sacs; a, abdomen; ES, egg sacs; ss, spermatophore sac; L4, 4th thoracic leg and UP, uropods.

Male:

The Body of male *L. kroyeri* is smaller than female (Fig. 24). Head (cephalothorax) is oblong and wider than long with antennal region set apart from rest of head. Genital complex indistinguishably fused to trunk. Abdomen of male is restricted and stumpy (Fig. 27). Caudal ramus (uropods) is long and slender (Fig. 27).

First antenna (Fig. 25) seven-segmented; first segment with one seta, second segment with three setae, third with short seta, fourth with one short and two long setae, fifth with one seta, sixth with two setae, seventh with eight terminal setae. Parabasal flagellum with broader base and is pointed distal part (Fig. 25).

Second antenna (Figs. 24 & 25) is sturdy and two-segmented, subchelate; corpus large, tapering distally with two processes on inner surface; subchela curving inwards with a spiniform process on inner surface close to the base.

Maxilliped (Figs. 24 & 26) is subchelate, corpus stout unarmed, subchela consisting of marginally denticulate shaft in male only, armed with single subterminal seta on inner margin and claw apicaly directed with longitudinal ridges.

First thoracic leg (Figs. 24 & 26) is biramous: with rows of denticles on outer margin of protopod and medial margin of endopod. Exopod is broad and distally armed with five terminal spines; endopod small, tapering distally, margins denticulate, with apical pilose seta longer than exopod. Sympod is denticulated, armed with spiniform process near base and medial to endopod.

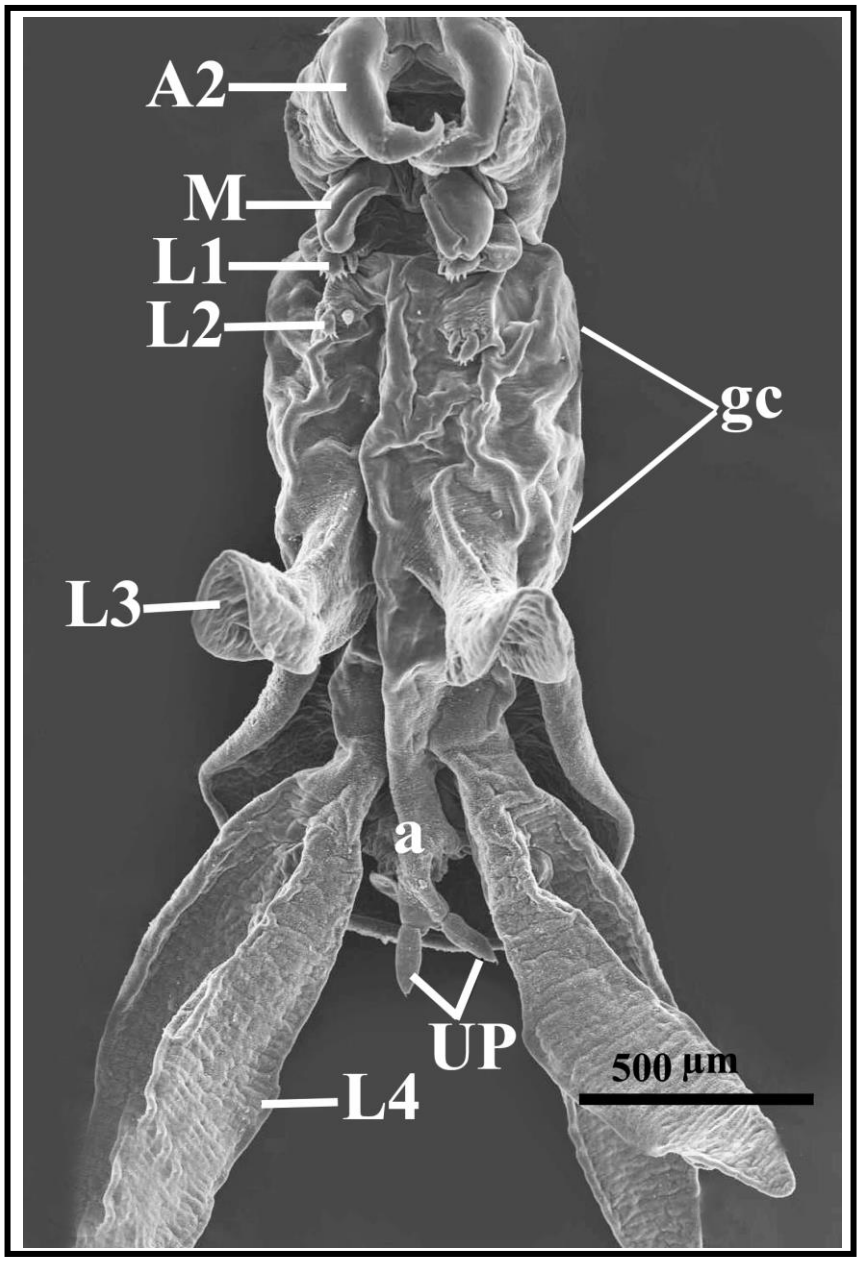


Figure (24): Scanning electron micrograph of adult male *Lernanthropus kroyeri* in ventral view; A2, 2nd antenna; a, abdomen; gc, genital complex; L1, 1st thoracic leg; L2, 2nd thoracic leg; L3, 3rd thoracic leg; L4, 4th thoracic leg; M, maxilliped and UP, uropods.

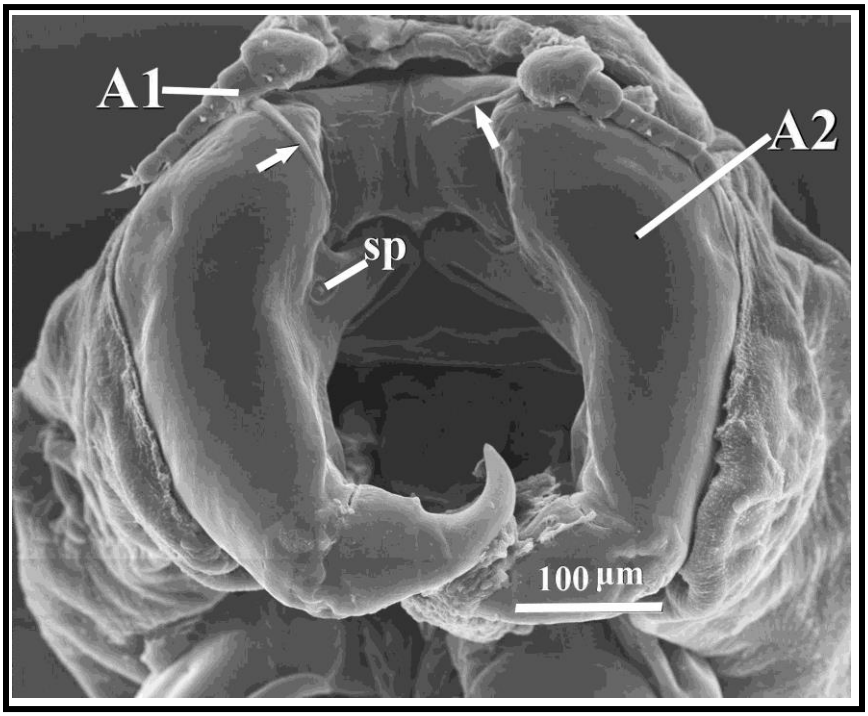


Figure (25): Scanning electron micrograph of adult male *Lernanthropus kroyeri* showing enlarged cephalothorax; A1, 1st antenna; A2, 2nd antenna and sp, spiniform process;. Arrows indicate parabasal flagellum.

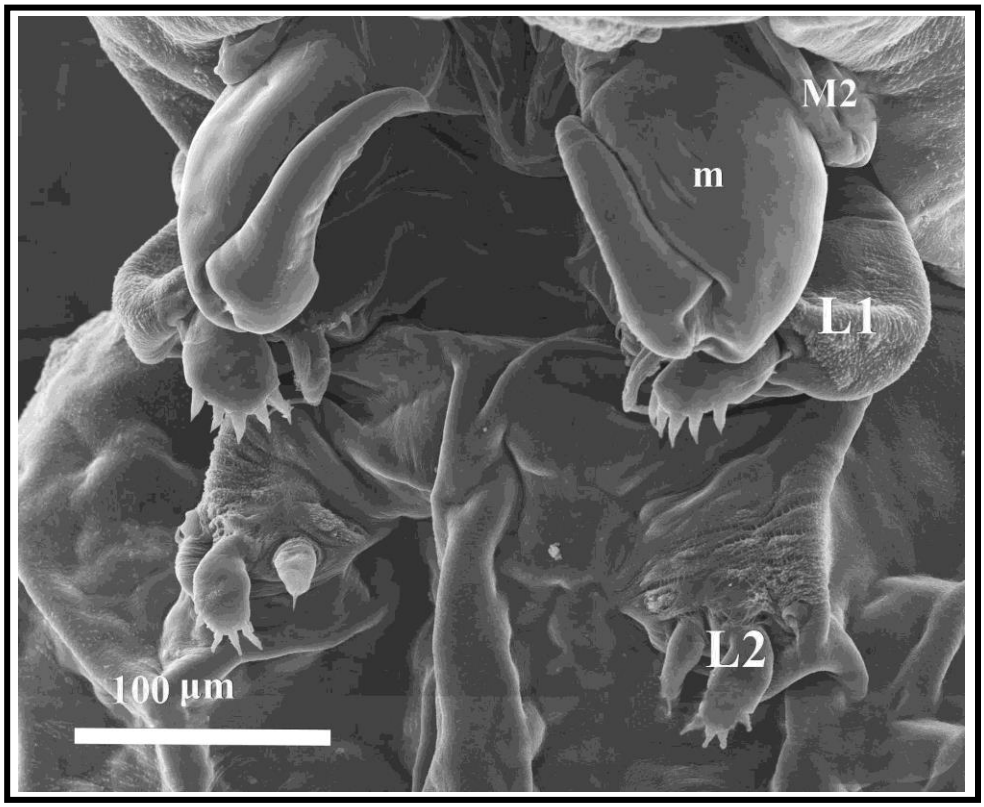


Figure (26): Scanning electron micrograph of adult male *Lernanthropus kroyeri* showing cephalothorax appendages; L1, 1st thoracic leg; L2, 2nd thoracic leg; M2, 2nd maxilla; m, maxilliped.

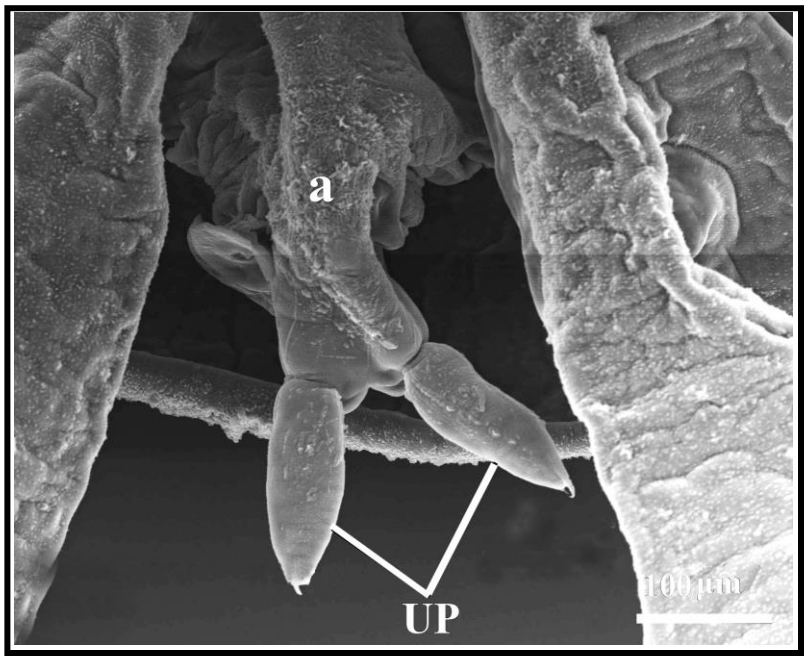


Figure (27): Scanning electron micrograph of adult male *Lernanthropus kroyeri* showing abdomen (a) and uropods (UP).

Second thoracic leg (Figs. 24 & 26) is biramous: exopod with five distal spines (naked- shape) and denticles covering distal base. Endopod with dense patch of denticles on medial surface and armed with short apical seta (naked in male). Sympod armed with naked seta, near base of exopod.

Third thoracic leg (Fig. 24) is long, thin and unarmed, protruding posteroventrally from medial region of genital complex, bilobed with long lateral lobe.

Fourth thoracic leg (Fig. 24) constructed as in leg 3, but is longer and armed with bifid denticles, protruding ventrolaterally from genital complex region.

Discussion:-

The sea bass are being infected by parasites due to environmental factors such as pollution and over storage. It causes stress on the fish making its body weaker, due to uncontrolled transfer of larva, obtaining the larva and breeding from natural sources. For these reasons, the intensive fish culture gave a great importance to study of the parasites and diseases that it causes. It is necessary, therefore to find out the parasite fauna in the aquatic environment. This will give us to apply the preventive practices on the fish in the fish farms.

The parasitic copepod in the present study agrees with that was studied by **Abu Samak (2004); Özel *et al.*, (2004)** and **Öktener *et al.*, (2010).** The availability of the material and the use of SEM of both sexes of *L. kroyeri* by **Abu Samak (2004)** have led to supplement the new information of *L. kroyeri* as follows: Presence of myxal process on the second antenna; of postantennal processes posterior to the second antenna in both sexes; of armature on both labium and labrum; of two buccal stylets; of 8 teeth on tip of the mandibles in both sexes and of single subterminal seta on inner margin of maxilliped subchela in both sexes; difference in type and density of cuticular processes covering the dorsal surface of cephalothorax between two sexes; in position of the first antenna setation between two sexes; in armature on postoral processes, first maxillae and claw of the second maxillae in both sexes; in number of myxal process on the second antenna in both sexes; in type of exopodal setation and denticles pattern covering first and second thoracic legs between two sexes; besides the male endopod of first thoracic leg with apical pilose seta longer than the exopodal length.

Also, **Abu Samak (2005)** observed that both sexes of parasitic copepod *Lernanthropus kroyeri* were attached to host tissue by cephalothoracic appendages including 2nd antennae, second maxillae and maxilliped. The present study indicates in detail shape of cephalothorax appendages; 1st antennae, 2nd antenna, 1st maxillae, 2nd maxillae, maxilliped, and the first to fourth legs.

According to **Abu Samak (2005)** the second antennae have the main and primary role during attachment while the second maxillae, maxillipeds and the third legs have secondary role during this process. Furthermore, Observation by **Ho *et al.*, (2011)** showed that lernanthropids used their prehensile antennae, maxillipeds and the third leg to attach on gill filaments.

Lernanthropus spp. are morphologically suited to attach to the host gill by means of the piercing action of the second antennae, which is assisted by the action of maxillipeds and the modified third pair of legs **Kabata (1979b).** The structure of the antenna was also seen under SEM with characteristically prehensile and uncinate which are used by the parasiteto attach or feed on the host tissue **(Kabata, 1985)**.

Compared with the previous results, the present study revealed that 1st maxillae bilobate with lobe smaller than outer ended by two terminal horny spines and setule cover and 2nd maxillae are uniramous, distally calamus claw armed with two sharp denticles rows, each comprising 13 teeth. Therefore, the 1st & 2nd maxillae may help the attachment of parasite onto host tissue.

Moreover, maxilliped is robust, terminal claw with longitudinal ridges and may have assistant role in attachment of parasite. Also,1st and 2nd thoracic legs are smaller and different in structure than previous appendages and ended with finger spines look like hand fingers. This structure led to attach with adjacent secondary gill lamellae and increasing the parasite stability. Besides that, 3rd and 4th thoracic

legs are the largest appendages, unique structure, foliaceous and without any adhesive sclerites or hooks. The shape of 3rd and 4th thoracic legs may suit with parasite habitat, serve to resist the water current and help the parasite position for tight attachment.

Ergasilus versicolor **Wilson, 1911**

1. General Topography:

The body of the female parasite is slender, elongated and gradually narrowed posteriorly. It is commonly known as the 'gill maggot' due to the presence of long white egg sacs that trail behind the body (Fig. 28). The parasite is usually attached towards the ends of the gill arches by the robust 2nd antennae leaving the medial filaments free, but this might not be true for all species.

2. Body form:

The body form of female parasite is long and narrow. The female body consists of four regions (Figs. 29 & 30):

1. Cephalothorax
2. Free thorax
3. Genital segment
4. Abdomen

Cephalothorax (Figs. 29 & 30) usually is longer than half total body, and lateral margins more or less parallel. The anterior end is partially flagging projecting antennary region and form short rostrum. The cephalothorax decreases in width posteriorly. Cephalothorax has one pair of antennules and one pair of antennae. The antennules (Fig. 31) are segmented and stumpy. Each one consists of six segments. The size of the segments is diminished distally towards the terminal end except the second segment which is slightly the biggest. All antennulary segments are provided with numerous simple setae, principally on

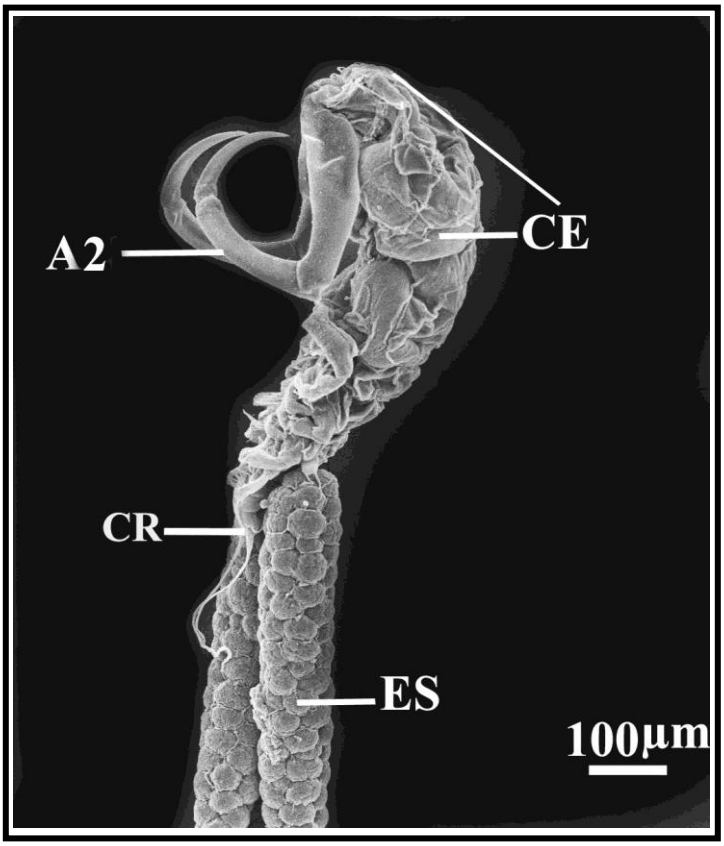


Figure (28): Scanning electron micrograph of adult female *Ergasilus versicolor* showing shape of the body; A2, 2nd antenna; CE, cephalothorax; CR, caudal ramus and ES, egg sac.

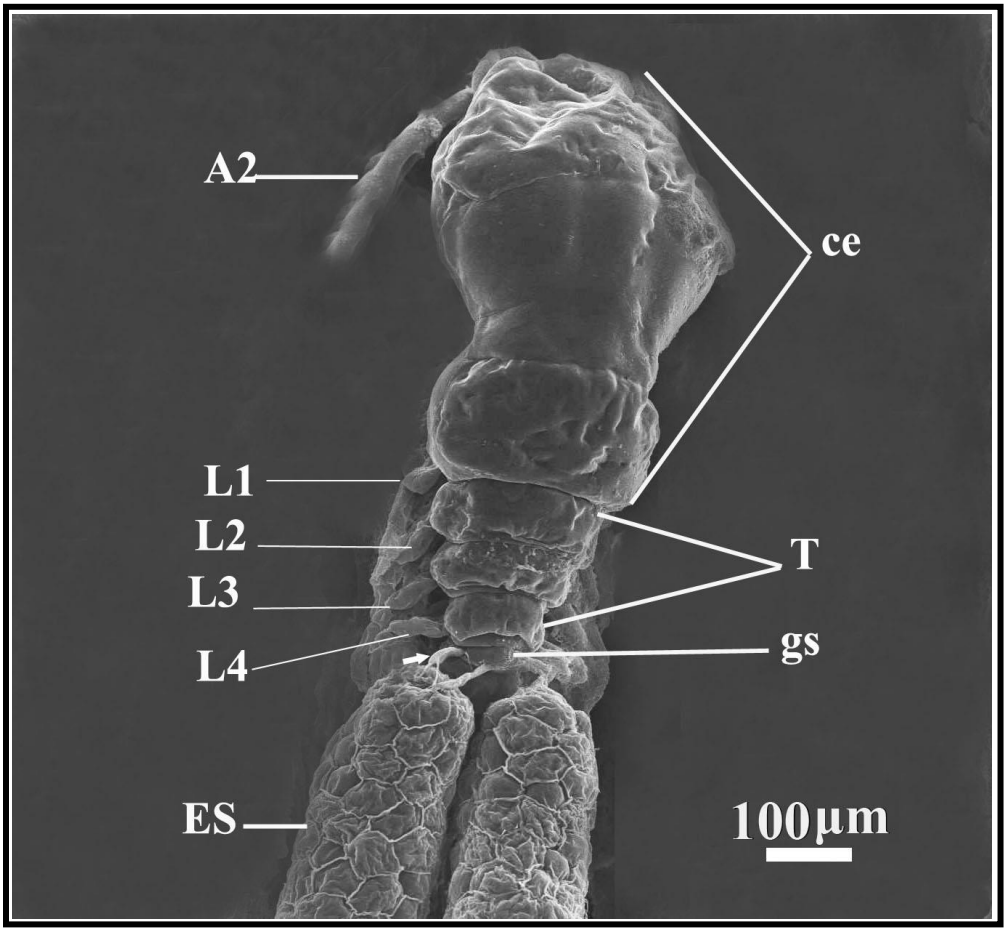


Figure (29): Scanning electron micrograph of adult female *Ergasilus versicolor* showing body form; A2, 2nd antenna; ce, cephalothorax; ES, egg sac; gs, genital segment; L1, 1st thoracic leg; L2, 2nd thoracic leg; L3, 3rd thoracic leg; L4, 4th thoracic leg and T, free thorax. Arrow indicates 5th thoracic leg.

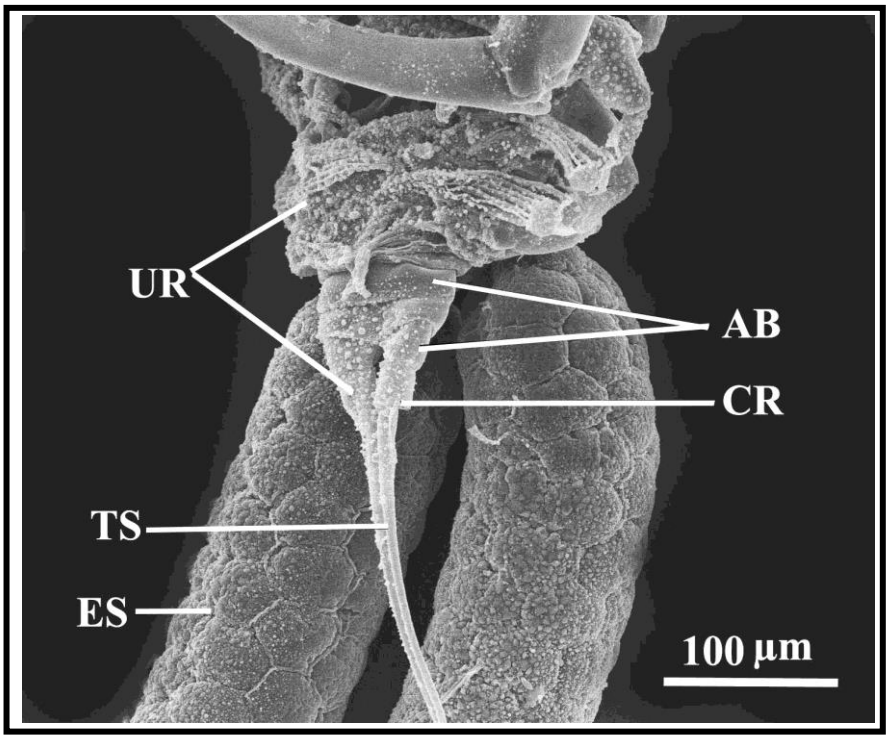


Figure (30): Scanning electron micrograph of adult female *Ergasilus versicolor* showing body form. AB, abdomen; CR, caudal ramus; ES, egg sac; TS, terminal seta and UR, urosome.

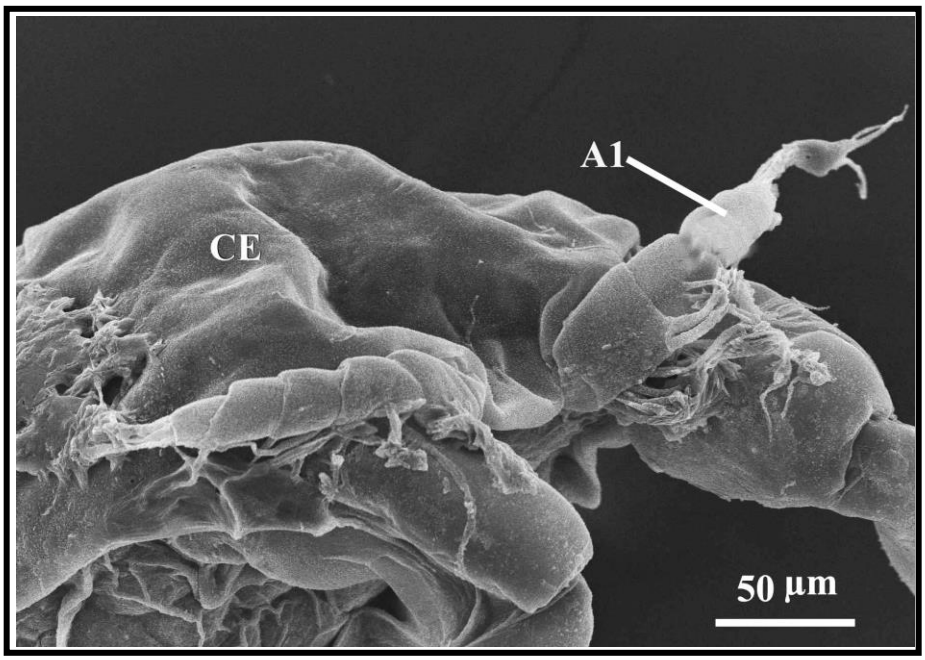


Figure (31): Scanning electron micrograph of adult female *Ergasilus versicolor* showing the anterior end of body, A1, 1st antenna and CE, cephalothorax.

antero-ventral surface. The number of setae on each segment starting from the proximal segment to the distal one is as follows; 3: 9: 3: 2: 2: 7. The number of these antennulary setae is known as “Setal formula” or “armature formula”.

The antennae (Figs. 28 & 32) are long, segmented and firm prehensile. Each one consists of four segments. The first or basal segment is the coxobasis. It is short, slightly cubical in shape. The second segment is the first endopodal segment which is the longest one, broad at its proximal end and tighten towards its distal end. The third antennary segment is the second endopodal segment and is termed the subchela that is narrow and arched with the sides almost parallel and clearly curved inwards. The last antennary segment is the fourth endopodal segment that ends with clasper-like smooth claw lacking tooth.

Free thorax contains five pairs of thoracic swimming legs (Fig. 29). The first pair of thoracic legs consists of a coxapod and basipod that ornamented on their anterior surface with rows of spinules. The basipod is ornamented also with a single antero-lateral sensilum. Each of exopod and endopod consists of three segments that diminish distally in size. The first exopodal segmental is serrated where there is one row of spinules. The second exopodal segmental is serrated laterally with one row of spinules arranged on its outer margin. The terminal exopodal on its outer margin. It is provided with two laterally situated serrated spines projecting from the outer margin. The endopodal segments are serrated where they are provided with one row of spinules arranged on the outer margins. The first endopodal segment is serrated also on the distal margin and provided with a single medially situated unarmed seta projecting from the inner segmental margin. The second endopodal segment is provided with a single postero-lateral naked seta. The terminal endopodal segment is provided with two serrated rasp-

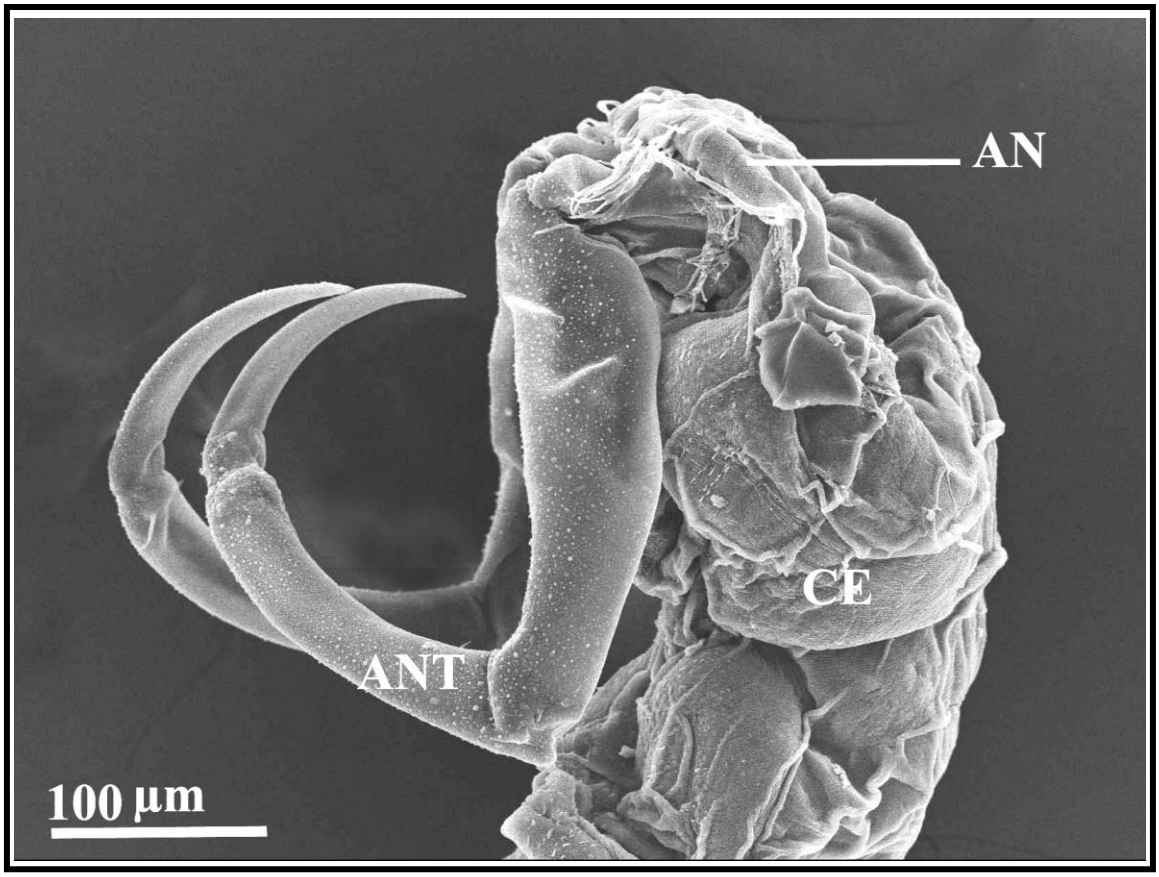


Figure (32): Scanning electron micrograph of adult female *Ergasilus versicolor* showing cephalothorax (lateral view), AN, 1st antenna; ANT, 2nd antenna and CE, cephalothorax.

like spine arranged on its outer margin in addition to four naked setae on both distal and inner segmental margins.

The second and third thoracic legs are closely similar (Fig. 29). The coxapod and basipod of each leg are ornamented on their anterior surfaces with rows of spinules arranged in scattered groups. The basipod of each leg is ornamented with a single antero-lateral sensilum projecting from the outer margin of the basipod. The exopod and endopod of both legs consist of three segments, diminishing distally in size. The first exopodal segment is provided with a single posterolateral rasp-like spine originates from its outer margin. The second exopodal segment is provided with a single medially situated unarmed seta located on the inner segmental margin. The terminal exopodal segment is provided with a single posterolateral rasp-like spine projects from the outer margin and moreover six posteriorly directed unarmed setae in which the outer is pectinate arranged on both distal and inner margins of the terminal exopodal segment. The endopodal segments are serrated where their outer margins are provided with a single row of spinules. The first endopodal segment is serrated also at distal margin and provided with a single medially situated unarmed seta located on the inner margin of the first endopodal segment. The second endopodal segment is provided with a single posterolateral unarmed seta projecting from the inner margin. The terminal endopodal segment is provided with a single posteriroly directed rasp-like projects from the distal segmental margin. Furthermore, it is provided with four posteriroly directed unarmed setae arranged on both distal and inner segmental margins.

The fourth thoracic leg (Fig. 29) consists of a coxapod that ornamented on its anterior surface with rows of spinules arranged in scattered groups and a basipod which ornamented also on its anterior surface with a single row of spinules. Also the basipod is provided with a single antero-lateral sensilum projecting from the

outer basipodal margin. The exopod consists of two segments while the endopod consists of three segments. Both exopodal and endopodal segments distally diminish in size and ornamented with a single row of spinules arranged on their outer and distal margin. The first exopodal segment is provided with a single posterolateral rasp-like; serrate spine which exists on the outer segmental margin. The terminal exopodal segment is provided laterally with a posterolateral rasp-like, serrate spine projecting from the outer segmental margin in addition to four posteriorly directed unarmed setae arranged on its distal margin; the outer one is pectinate. The first endopodal segment is ornamented laterally and inwardly with medially situated unarmed seta. The second endopodal segment is provided with a single posterolateral unarmed seta which exists on the inner segmental margin. The terminal endopodal segment is provided laterally with a single serrate, rasp-like spine located on the outer segmental margin besides three posteriorly directed unarmed setae arranged on the distal segmental margin.

Finally, the extremely reduced fifth thoracic swimming leg (Fig. 29) is represented by papillary process ornamented with a single posterolaterally directed unarmed sensilum located on the distal end.

The urosome (Fig. 30) consists of the two last thoracic somites (fifth and sixth), the abdominal somites and the caudal rami. The fifth thoracic somite is extremely reduced, very small and short but distinctly apart as much from the fourth thoracic segment as from the genital segment. The sixth thoracic somite is the genital somite.

The abdomen (Fig. 30) consists of three somites. These somites are wider than long, with almost similar width and slightly diminish posteriorly. The first abdominal somite is larger than the following two. The second abdominal somite is notched posteriorly almost up to the half of its length. The last somite is almost

equal to or slightly smaller than the second one. Each forked part of the third abdominal somite carries a single caudal ramus (Fig. 30). The length of each ramus is almost three times longer than its width, almost equal to the last two abdominal segments. Each caudal ramus is armed distally with four terminal setae. The innermost one is the largest, the outermost seta is shorter than the innermost one and the middle two are ventrally located. They are small and almost unnoticeable from the dorsal side.

3. Prehension:

The female *Ergasilus versicolor* attaches by means of the second antennae (Fig. 32). These antennae have powerful muscles and roughened surfaces. There are no lunules or sucking disks present in this genus. The body of the parasite always lies parallel with the gill filament, with the head of parasite towards the base of the filament or gill arch.

Discussion:-

Gill lice, a parasite of the genus *Ergasilus*, is a host-specific ectoparasite that infects many species of freshwater fish including yellow perch, walleye, brook trout, salmon, and large and small mouth bass **(Roberts 1970)**. Ergasilids are generally fresh-water copepods but some of them are marine. Eggs of the gill lice are in external egg sacs attached to the parasitic female. Once developed they hatch and the nauplii, (the first larval stage of many copepods) become free-living.

Gill lice of the genus *Ergasilus* go through several copepodid stages (second larval stage) following various nauplii stages. After reaching maturity, adults mate and, while males remain free-living, females become parasitic **(Hudson and Lesko 2003).** Body terminology follows **Kabata (1992), El-Rashidy and Boxshall (2001)** and **Montu and Boxshall (2002).** All descriptions refer only to female copepods, as male and larval stages are free living and their morphology is not known.

The parasitic copepod *Ergasilus versicolor* was firstly proposed by **Wilson, 1911** on catfishes, bluegill and striped mullet in the USA, **Williams (1994).** It could also be a native parasite that occurs on mullet, **Williams (1994).** The new species of *Ergasilus* described in this study is the first record in Egyptian Mediterranean coast of this genus on *Mugil cephalus*. **Siquier (2012)** described it from the gills of mullet fishes, *Mugil platanus* in from Uruguay.

Baker (2011) collected it from catfish**,** *Ictalurus punctatus* from Western Lake Erie in USA. The female parasite is capable of moving from one filament to the next with the aid of the four well developed pairs of swimming legs. The parasite can move forward towards the branchial arches by creating a vacuum with the cephalothorax, when the vacuum is broken the parasite can push itself

forward. Also, this might be necessary for feeding purposes. It has been known that Ergasilidae exhibit the primitive cyclopoid morphology with few but effective, adaptations for parasitism **(Wilson 1911, Kabata 1979a** and **Cressey 1983).**

In the present study, a careful comparison of the present specimens with those described by previous authors revealed that, they are related to the specimens redescribed by **Kabata (1992)** in the structure of antennule and antenna, shape of cephalothorax, free thoracic segments, width of abdominal segments, caudal rami setation, swimming legs. Meanwhile, the present specimens differ from those described by **Kabata (1992)** in width of free thoracic segments and length of genital segment.

According to **Siquier (2012)** *E. versicolor* individuals were found strongly attached by the antennae, in a parallel position with respect to gill filaments, and the cephalosoma directed towards the base of the gill arch. Furthermore, observation under SEM microscope by **Baker (2011)** showed that ergasilides used their prehensile antennae and the thoracic legs to attach on gill filaments.

Compared with the previous results, the current study suggested that the appendages topography of *E. versicolor* under SEM could be explained by the parasites firmly attaching themselves to the gill filaments with their second pair of antennae to prevent them from being washed away. This property allows to this species to occupy the largest, most oxygenated and mucus-rich areas.

We further observed that only the 2nd antenna plays a role in the primary attachment to gill filaments under SEM. The assistant action of attachment is made by the four swimming legs.

In conclusion, *Ergasilus versicolor* under discussion is highly adapted in its parasitizing site, where it is armed with different attachment structure; second antennae and swimming legs. All the former structures help the parasite in facing the strong water current inside the fish gills.

Part 1

Morphological and Anatomical studies

Transmission electron microscope Studies

The Tegument

a- *Lernanthropus kroyeri*

In semi-thin sections stained with toluidine blue revealed that dorsal surface of the body tegument shows two types of epicuticular formations:

1- Very abundant ones with a tubular or filamentous aspect.

2- The other type of cuticular outgrowths consists of longer and more ramified expansions less abundant than the other ones.

The cuticle is thick and shows three different zones : epicuticle, exocuticle, and endocuticle (Figs. 33, 34, 35 & 36). It is crossed by a great number of canalicles, which allow the passage of substances from the underlying epithelium (Fig. 35). A well-defined epicuticle was present in all sections studied. The epicuticle was always covered by a fuzzy coat, having the appearance of a mucoid layer. Epidermis have the typical morphology of this integumentary tissue, and the two main features are: a great number of septate junctions and mitochondria with very developed and abundant cristae (Fig. 36).

Observations using transmission electron microscope have revealed that the cuticle is underlain by a single-layered epidermis which overlies integumental glands and chromatophores (Figs. 37, 38 & 39). The cuticle was distinguished by the presence of alternating light and dark bands and by the appearance of a fibrous structure through most of its depth (Fig. 36).

The base of the endocuticle in contact with the epidermis was less well organised than the overlying laminated cuticle, often possessing a more granular appearance and having small electron-lucent inclusions. The epidermis comprised a single layer of cells separated from the endocuticle by an electron-dense apical

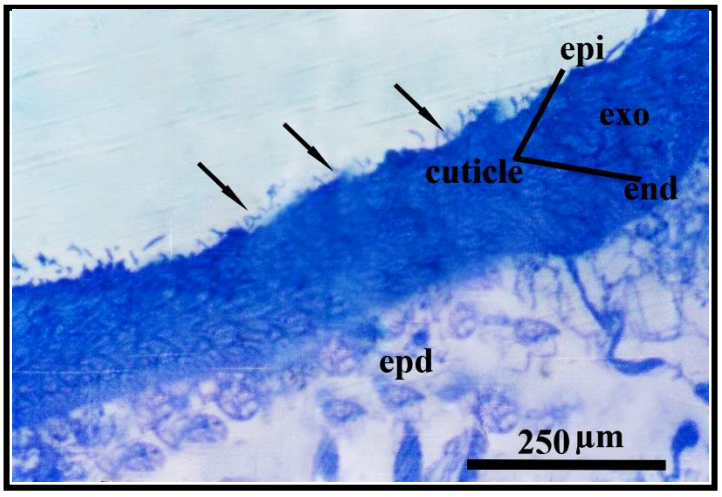


Figure (33): Photomicrograph of a semi-thin, toluidine blue-stained section through female *Lernanthropus kroyeri* showing its tegument (Dorsal view). Note: cuticular outgrowths (arrows); end, endocuticle; epd, epidermis; epi, epicuticle and exo, exocuticle.

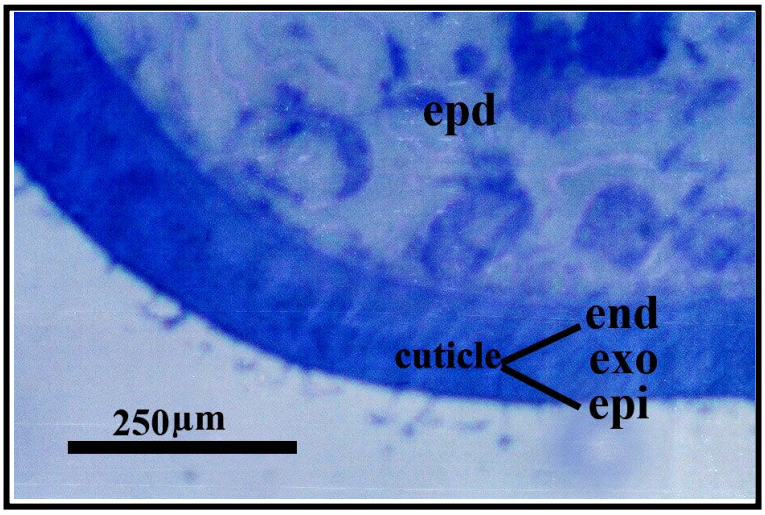


Figure (34): Photomicrograph of a semi-thin, toluidine blue-stained section through female *Lernanthropus kroyeri* showing its tegument (ventral view). Note: end, endocuticle; epd, epidermis; epi, epicuticle and exo, exocuticle.

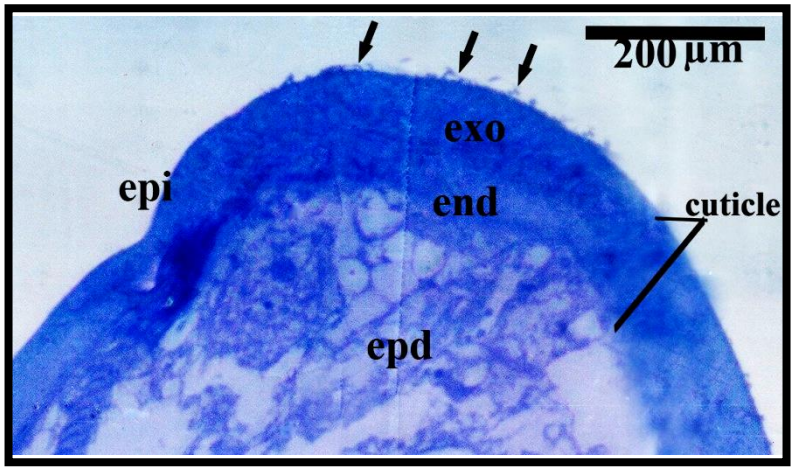


Figure (35): Photomicrograph of a semi-thin, toluidine blue-stained section through male *Lernanthropus kroyeri* showing its tegument (dorsal view). Note: cuticular outgrowths (arrows); end, endocuticle; epd, epidermis; epi, epicuticle and exo, exocuticle.

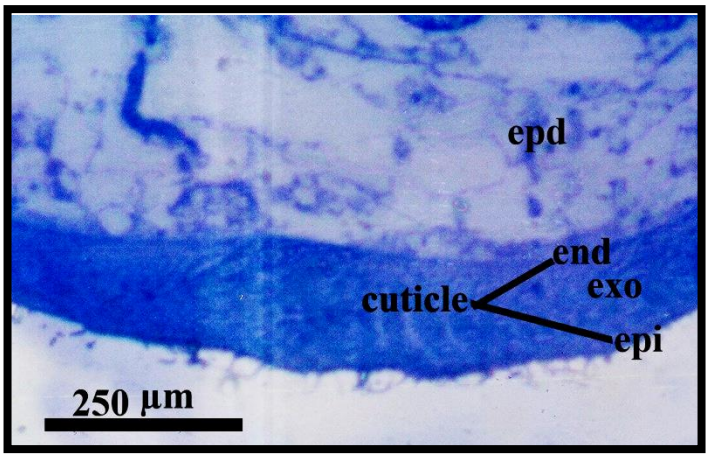


Figure (36): Photomicrograph of a semi-thin, toluidine blue-stained section through male *Lernanthropus kroyeri* showing its tegument (ventral view). Note: end, endocuticle; epd, epidermis; epi, epicuticle and exo, exocuticle.

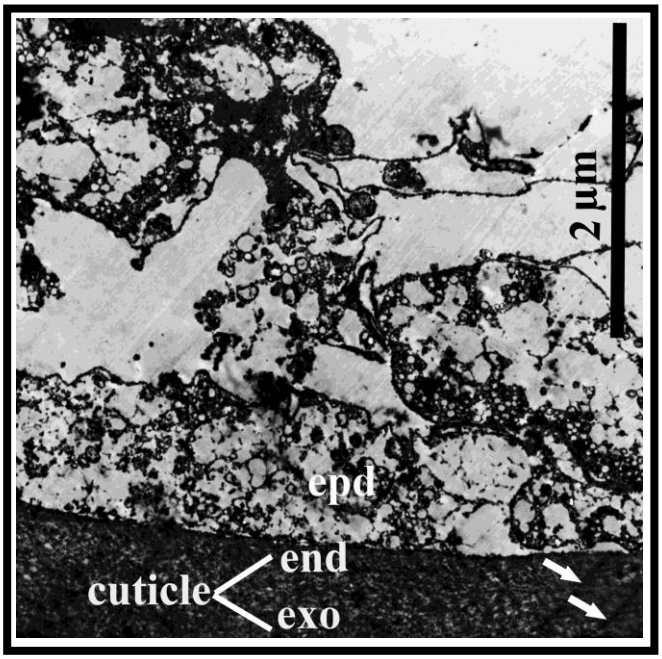


Figure (37): Transmission electron micrograph showing body wall layers of female *Lernanthropus kroyeri* (ventral view). Note: some cross canalicles, (arrows); endocuticle (end); epidermis (epd) and exocuticle (exo). (X 1000).

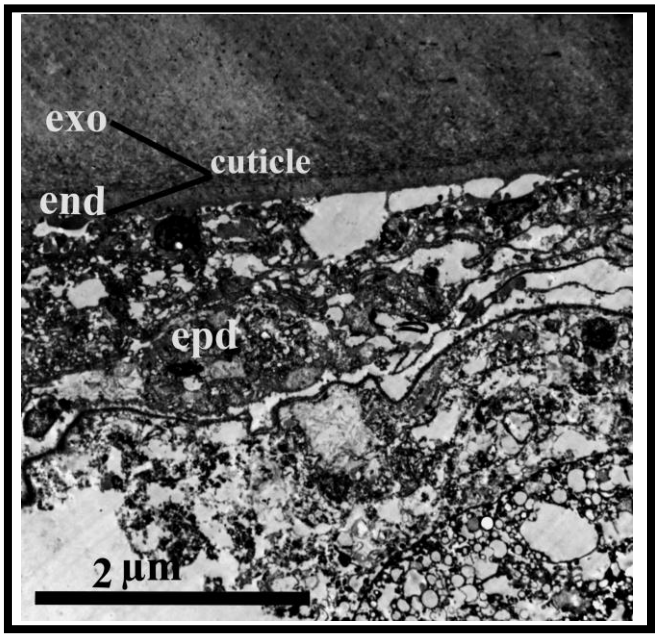


Figure (38): Transmission electron micrograph showing body wall layers of female *Lernanthropus kroyeri* (dorsal view). Note: endocuticle (end); epidermis (epd) and exocuticle (exo).

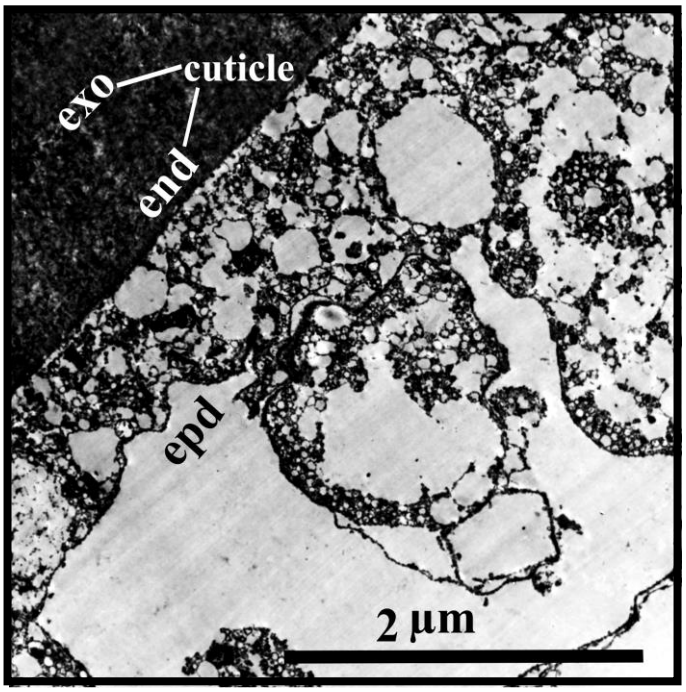


Figure (39): Transmission electron micrograph showing body wall layers of male *Lernanthropus kroyeri* (dorsal view).
Note: endocuticle (end); epidermis (epd) and exocuticle (exo). (X 1000).

membrane which was often elaborated into rugose folds. TEM observations have revealed that the cuticle of the dorsal body surface was generally thicker than that of the ventral body surface (Figs. 35, 36 & 37). The cuticle depth varied, was up to 3.5 µm thick in dorsal surface and 2.4 µm in ventral surface.

b- ***Ergasilus versicolor***

The cuticle consisted of three recognisable zones (Fig. 40). These comprised a multi-layered external epicuticle, exocuticle and an internal endocuticle from outermost to innermost. The cuticle of the dorsal body surface was generally thicker than that of the ventral body surface (Figs. 40 & 41). A well-defined epicuticle was present in all sections studied. The epicuticle was always covered by a fuzzy coat, having the appearance of a mucoid layer.

Observations using transmission electron microscopy have revealed that in *Ergasilus versicolor* the chitinous outer layer of the body wall is thick throughout the whole body and was up to 2 µm thick (Figs. 40, 41 & 42). The body wall is equipped with a very strong chitinous layer and a thick epidermis (Figs. 40, 41 & 42). The cuticle in all of these body areas is flexible. There was no indication of any cuticular outgrowths or papillae of cuticular surface (Figs. 40 & 41).

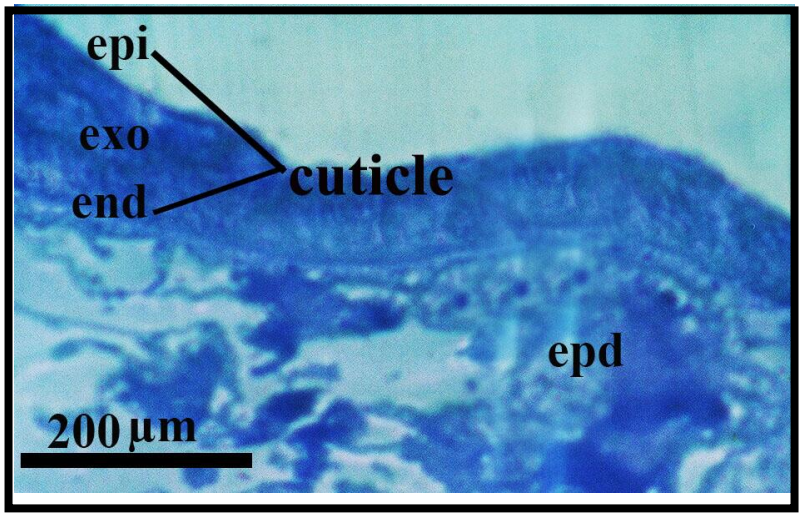


Figure (40): Photomicrograph of a semi-thin, toluidine blue-stained section through female *Ergasilus versicolor* showing its tegument (dorsal view). Note: end, endocuticle; epd, epidermis; epi, epicuticle and exo, exocuticle.

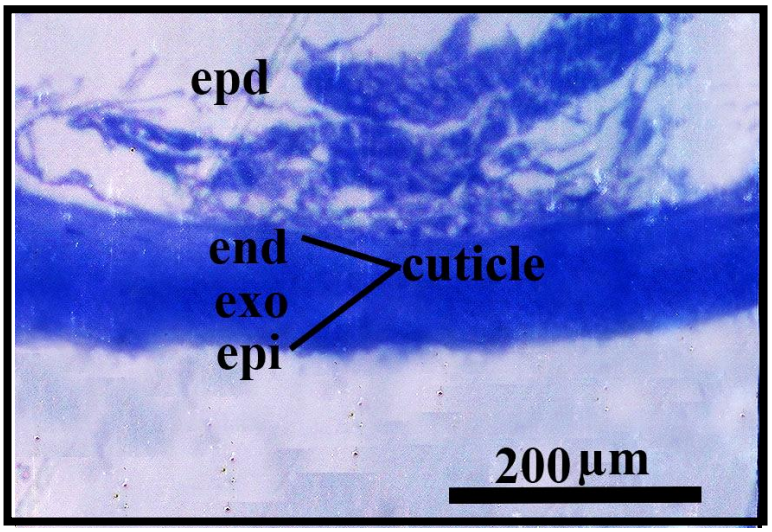


Figure (41): Photomicrograph of a semi-thin, toluidine blue-stained section through female *Ergasilus versicolor* showing its tegument (ventral view). Note: end, endocuticle; epd, epidermis; epi, epicuticle and exo, exocuticle.

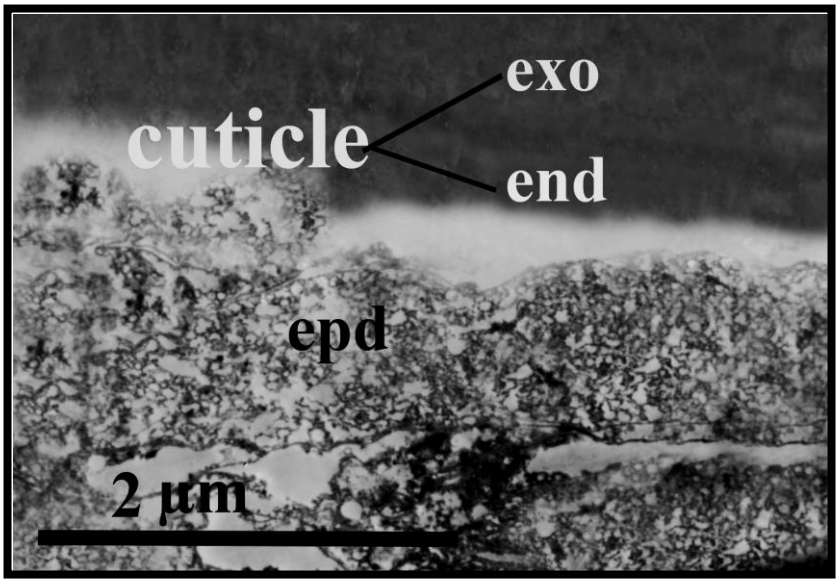


Figure (42): Transmission electron micrograph showing body wall layers of female *Ergasilus versicolor* (dorsal view).
Note: endocuticle (end); epidermis (epd) and exocuticle (exo). (X 1000).

Discussion:-

Comparing with other crustacean groups, parasitic copepods have the most various structures and lifestyles (**Razouls 1996; Lee *et al.*, 2005**). Moreover, they have extraordinary differentiations and morphological regressions (**Cabral *et al.*, 1984**). Tegument, surface morphology, especially the number, shapes, size, and distribution of various tegumental structures may be helpful to understand the life and physiological condition of parasites. *Lernanthropus kroyeri* Van Beneden is an ectoparasite of *Dicentrarchus labrax* which is found attached to the gill filaments. Due to this fact, it shows morphological modifications at different levels, which are studied through transmission electron microscopy. The present study gives new and additional morphological information on both sexes of *Lernanthropus kroyeri*.

Recent studies have revealed the remarkable diversity of parasitic copepod sensory abilities and behaviors. Body surface of parasites is richly equipped with numerous receptors, most of them with functional significances, monitoring chemical and mechanical signals from surrounding environment **(Gresty *et al.*, 1993** and **Heuch *et al.*, 2007).** Several types of sensory structures identified on *L. kroyeri* were previously observed in copepods, although the form and distribution varies according to species (**Boxshall *et al.*, 1997**).

Numerous sensory endings identified on body surface of *L. kroyeri* are involved with feeding and attachment. Also, provided the copepod with a considerable increase of cuticular surface in order to a better oxygen utilization, and so improving respiratory processes through the integument (**Antonelli, 2012**).

Cuticular differentiations play a secondary role in the fixation and they have mainly a sensory function. The cuticle of *Lernanthropus kroyeri* provides the principal interface between the organism and its external environment. Amongst other functions it acts as a defence against pathogens / host attacks, constitutes a barrier mediating osmotic and respiratory exchanges and provides a site for support / attachment of the body musculature and internal organs (**Antonelli, 2012**).

In this study semithin sections of *L. kroyeri* revealed that presence of uniciliated sensory structures, occur regularly on all body surfaces, except on antennae. These receptors show variation in distribution and morphology. They may occur singly or in groups. Long fine, dense, and intermingled, they are observed on dorsal body surface.

On the other hand, semithin sections of *E. versicolor* showed that there is no outgrowth or sensory structures on the body surfaces. It seems likely that these structures that not observed of *E. versicolor* in the present study were similar to those reported for other Ergasilids by **(Salmen *et al.*, 2008** and **Schrodl 2013)** and revealed that the papillae or other outgrowth structures of *Ergasilus aliena* on the body surface or related to respiration could not be detected.

This indicates that the entire body surface is involved in exchange of gases, confirming earlier assumptions. In the present study semi-thin sections stained by toluidine blue, the ergasilid parasites have bodies which are much thickened in proportion to their breadth; furthermore, both the ovaries and oviducts are developed inside the cephalothorax.

In conclusion, the current study appeared that the tegument layers of *Lernanthropus kroyeri* and *Ergasilus versicolor* were divided into two layers: cuticle and epidermis. The cuticle is thick, shows three markedly different zones: epicuticle, exocuticle, and endocuticle. The structure of cuticle layer of *Lernanthropus kroyeri* is very similar to that described by (**Antonelli, 2012**). Also, the cuticle layer of *Ergasilus versicolor* in the present study like to that appeared with (**Bannister, 1993).** Moreover, the current study revealed that the cuticle of *Lernanthropus kroyeri* was thicker than that of *Ergasilus versicolor*. The epidermis was relatively simple, having a single layer of cells corresponding to most other described copepods and obviously for stability and protective reasons.

Part 2

Aspects of the pathology and infection of *Lernanthropus kroyeri* and *Ergasilus versicolor*

Normal histology of the gills

Under light, SEM and TEM microscopy, the gill morphology of uninfected specimens of *Dicentrarchus labrax* and *Mugil cephalus* were illustrated.

A- *Dicentrarchus labrax*

i- Light microscopy studies

There was an interbranchial median elevation appeared in sea bass. The gill arches length and the gaps between decreased medial wards in the fish. The gill rakers had similar arrangement and varied number in the fish. The first lateral rakers row was the longest one in sea bass. However the gill filaments appeared in double rows. Gill filaments were long at middle and short at extremities of gill arch **(Alsafy, 2013).**

The gill hemibranch is made up of double rows of gill filaments (primary gill lamella) from which arise perpendicularly the gill lamellae (secondary gill lamellae) which are thin walled and represent the site of gaseous exchange between the contained blood and the water flowing through the gill (Fig. 43). The primary gill lamella is supported by a median cartilaginous ray which runs along its longitudinal axis. The cartilaginous ray consists of a thick central band consisting of large irregularly shaped cartilaginous cells or chondrocytes. The latter is surrounded by a thin layer of fibrous coat formed of connective tissue and outlined by a single layer of simple squamous epithelium that consists of spindle-shaped or flattened cells (Fig. 43).

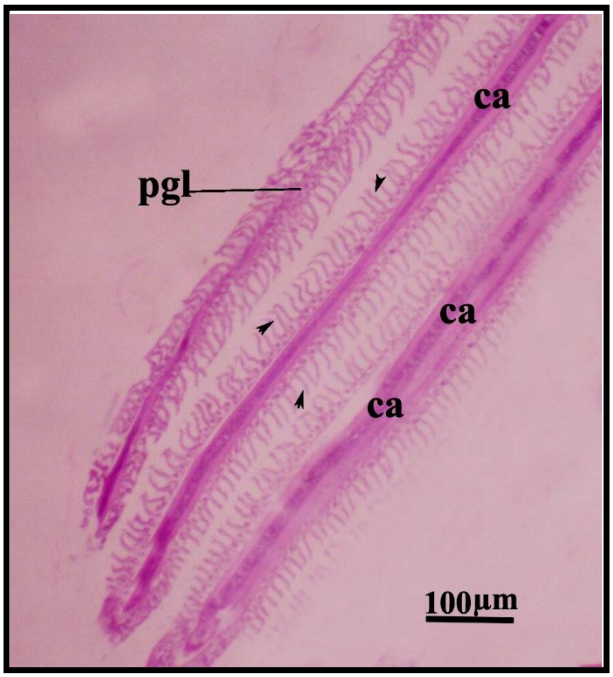


Figure (43): Light photomicrograph of non-infested (control) gill filament of *Dicentrarchus labrax* stained with haematoxylin and eosin showing the secondary gill lamellae (arrowheads) projecting on both side of primary gill lamella (pgl); ca, cartilage.

ii- semithin observation of gill structure

In semi-thin sections stained with toluidine blue, each gill arch bears a double row of primary gill filaments (non respiratory lamellae), and each filament carries two rows of secondary gill lamellae (respiratory lamellae).The gill filament lined by a thick stratified epithelium referred as primary epithelium. The secondary gill lamella (respiratory lamella) is lined on both opposite sides by an epithelium that is two squamous cells layer thick (Fig. 44).

iii- Scanning electron microscopy studies

Scanning electron microscopy revealed the surfaces of gill arch covered by a mosaic of pavement cells, varied sized pores of chloride cells and many pointed spines on the rakers ventral border in the sea bass **(Alsafy, 2013)** (Fig. 46). There are taste buds marked on the gill arches and rakers in sea bass Many lamellae appeared on filaments in the sea bass. The secondary gill lamellae (Fig. 45) are thin walled and represent the site of gaseous exchange between the contained blood and the water flowing through the gill.

iv- Transmission electron microscopy studies

The ultra-structural study on the gills of *Dicentrarchus labrax* showed that the secondary gill lamella (respiratory lamella) is lined on both opposite sides by an epithelium that is two layers of squamous cells in thick. The external layer is characterized by the fiat elongated pavement cells. (Figs. 47 & 48). Mucous cells are large modified columnar epithelial cells. They are found at the surface between other epithelial cells of the primary gill epithelium and at the base of secondary gill lamellae (Fig. 47). The mucous cells open to the external medium

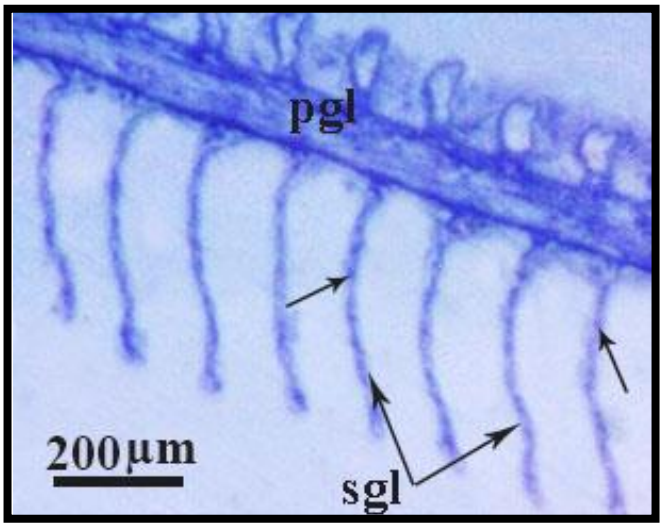


Figure (44): Photomicrograph of a semi-thin, toluidine blue-stained section through a primary gill filament (pgl) of a non-infested *Dicentrarchus labrax* and secondary gill lamellae (sgl), Arrows indicate pillar cells.

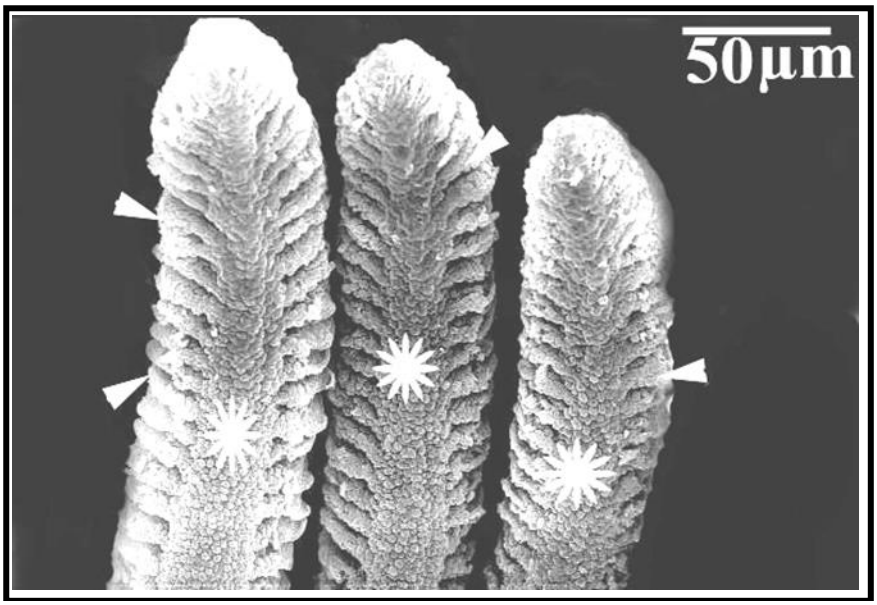


Figure (45): Scanning electron micrograph showing the normal topography of primary (*) and secondary lamellae (arrowheads) on the distal portion of the filaments of *Dicentrarchus labrax*.

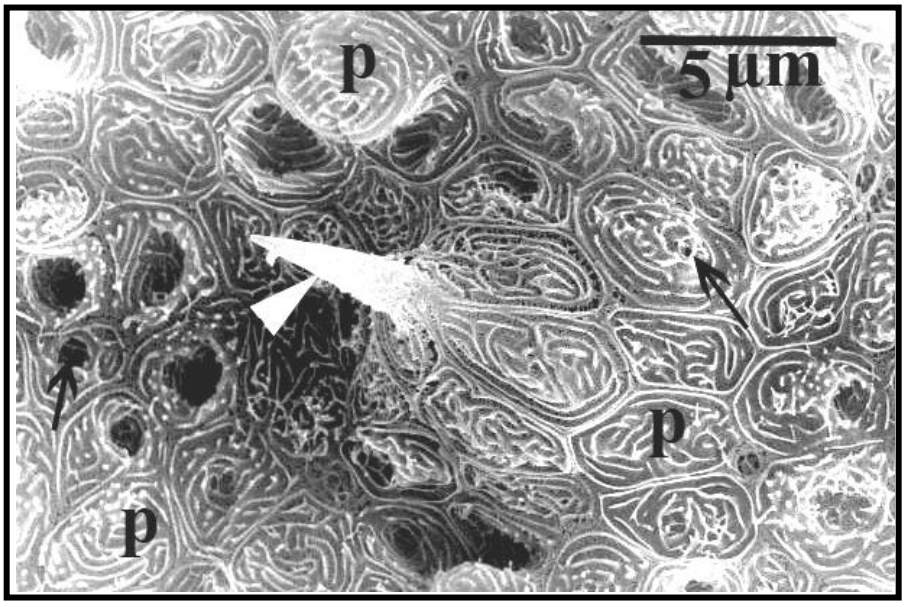


Figure (46): Scanning electron micrograph showing the surfaces of gill arch of *Dicentrarchus labrax*. Note: varied sized pores of chloride cells (arrows); pavement cells (p) and spine (arrowhead) on the rakers.

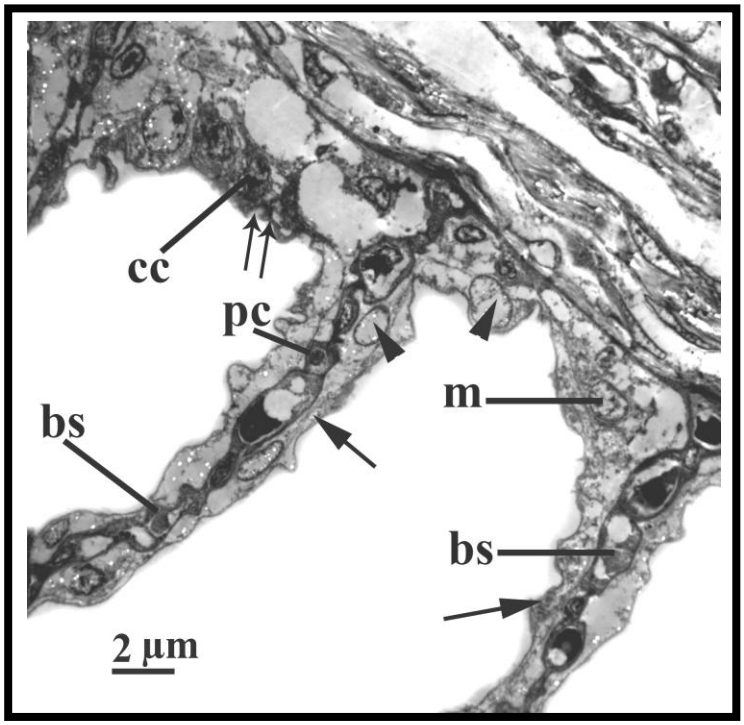


Figure (47): Transmission electron micrograph through a primary gill filament of a non-infested *Dicentrarchus labrax* showing secondary gill lamellae (large arrows). Note, apical pits (small arrows) of the chloride cells; blood spaces (bs); the chloride cells (cc); mucous cells (m); the flat elongated pavement cells (arrowheads) and pillar cell (pc). (X 500).

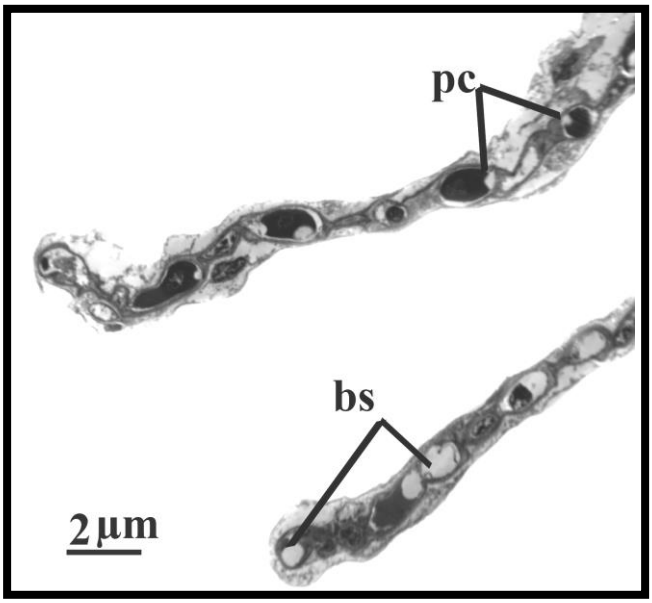


Figure (48): Transmission electron micrograph showing a distal part of the secondary gill lamellae of a non-infested *Dicentrarchus labrax*. Note, blood spaces (bs) and pillar cells (pc). (X 500).

with a deep apical pit which is surrounded by extensions of adjacent pavement cells. Small cytoplasmic processes from the mucous cells extend between adjacent epithelial cells (Fig. 47).

Observations using transmission electron microscope have revealed that chloride cells are large, polygonal, rounded, ovoid or columnar in shape, irregularly spaced and usually covered by superficial pavement cells. They are characterized by a rich population of mitochondria evenly distributed throughout the cytoplasm, which are variable in size and shape. A conspicuous feature of many chloride cells is the presence of an apical pit opening to external environment. The pit is usually located between two superficial pavement cells. The pit may contain finely granulated material (Fig. 47). The other chloride cells which were not in contact with the external medium had sheets of pavement cells covering their apical surfaces.

B- *Mugil cephalus*

i- Light microscopy studies

The gill filaments are supported by a complete interbranchial septum and water exits via external branchial slits or pores. In contrast, the Mugilidae interbranchial septum is much reduced, leaving the ends of the filaments unattached, and the multiple gill openings are replaced by the single caudal opening of the operculum. The primary gill lamella is supported by a median cartilaginous ray and protruding from it the secondary gill lamellae (Fig. 49).

The basic functional unit of the gill is the filament, which supports rows of plate-like lamellae. The lamellae are designed for gas exchange with a large surface area and a thin epithelium surrounding a well-vascularized core of pillar

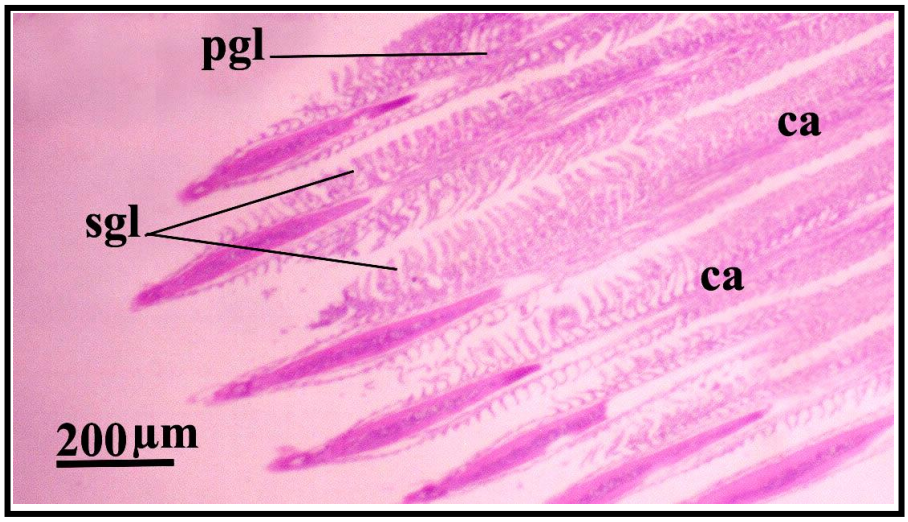


Figure (49): Light photomicrograph of non-infested (control) gill filament of *Mugil cephalus* stained with haematoxylin and eosin showing the secondary gill lamellae (sgl) projecting on both side of primary gill lamella (pgl); ca, cartilage.

cell capillaries. The lamellae are positioned for the blood flow to be counter-current to the water flow over the gills.

ii- semithin observation of gill structure

In semi-thin sections stained with toluidine blue The gill lamellae resembled flattened sacs perpendicular to the axis of the gill filament. Both structures were found to be covered by a specialized epithelial lamina according to their locality: a stratified epithelium on the filaments; and a simple epithelium on the gill lamellae. Various types of cells were identified on the epithelial lamina: superficial pavement cells, mucous goblet cells, undifferentiated cells and chloride cells. The chloride cells were most frequently observed in interlamellar regions of the filament and at the base of the lamellae (Fig. 50).

iii- Scanning electron microscopy studies

The surface ultrastructure of the gill arches of the mullet, *Mugil cephalus*, was studied by scanning electron microscopy. Each gill arch supports two rows of gill filaments which extend posterolaterally, and two rows of gill rakers which extend anteromedially **(Hossler *et al.*, 2005)**. On the mullet gill arches, taste buds were limited to the pharyngeal surfaces of the smooth-surfaced gill rakers, **Hossler and Merchant (2005)**. Secondary lamellae (respiratory lamellae) extend from both lateral surfaces of each filament (Figs. 51 & 52), and numerous secondary projections, either spiny or smooth, extend from the anteromedial aspect of each raker. Raker morphology varies somewhat from one gill arch to the next and from the dorsal to ventral aspects of the same gill arch. All surfaces of the gill arch except the respiratory lamellae and portions of the rakers are covered with a mosaic of ridged epithelial cells.

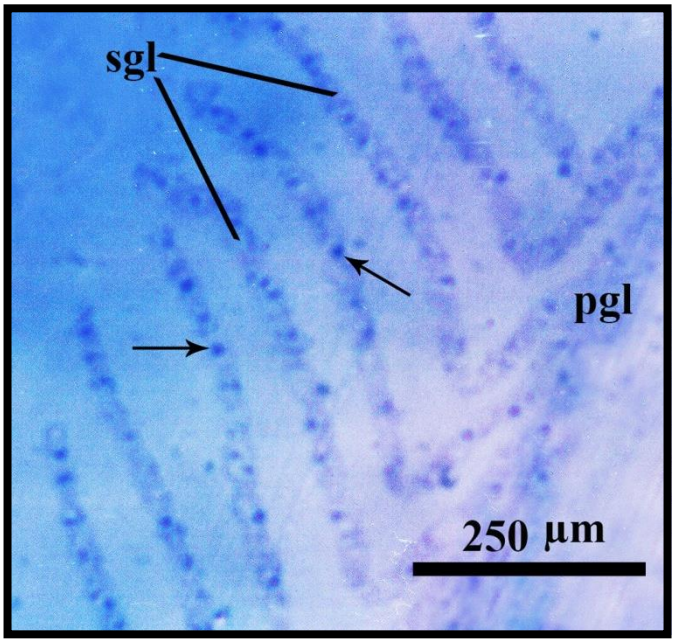


Figure (50): Photomicrograph of a semi-thin, toluidine blue-stained section through a primary gill filament (pgl) of a non-infested *Mugil cephalus* and secondary gill lamellae (sgl), Arrows indicate pillar cells.

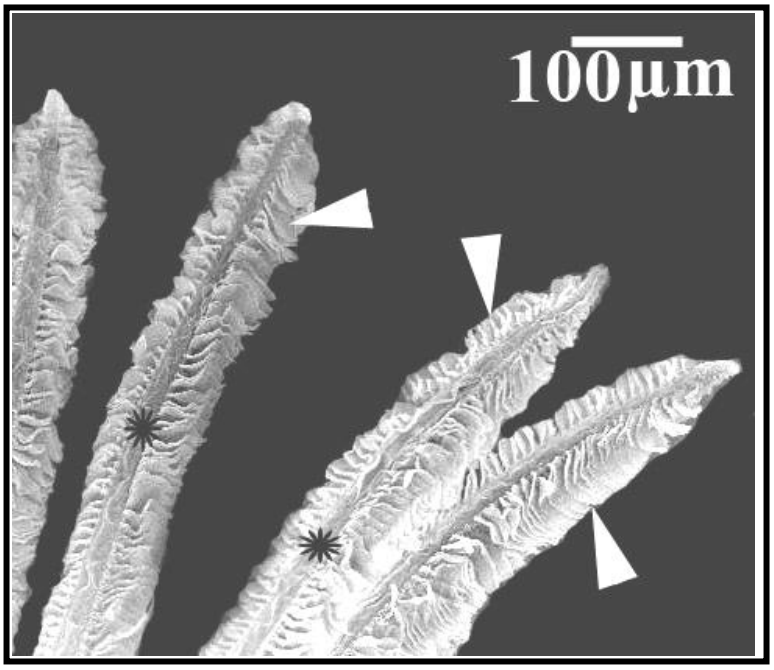


Figure (51): Scanning electron micrograph showing the normal topography of the gill filaments of *Mugil cephalus*. Note, the primary gill lamellae (*), the secondary gill lamellae (arrowheads).

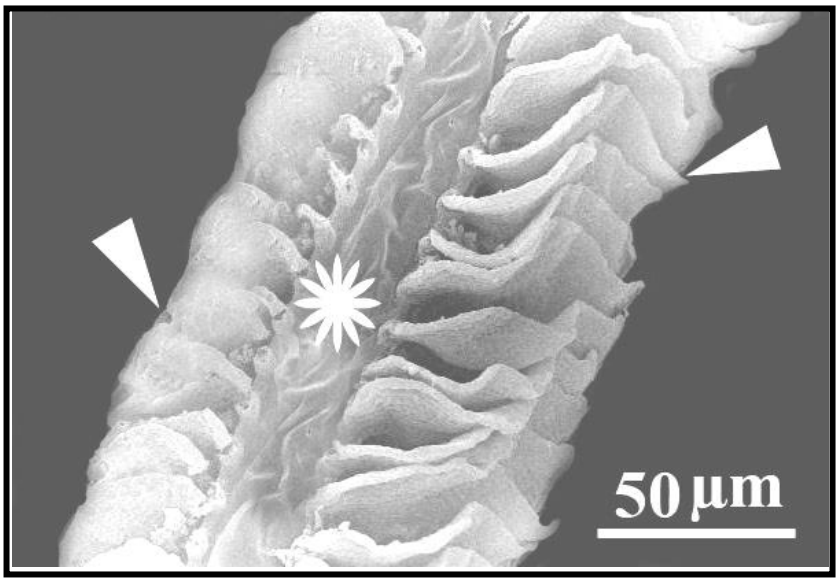


Figure (52): Scanning electron micrograph showing the normal topography of part of gill filament of *Mugil cephalus*. Note, the primary gill lamellae (*), the secondary gill lamellae (arrowheads).

iv- Transmission electron microscopy studies

The ultra-structural study on the gills of *Mugil cephalus* showed that the branchial filaments are shorter on the extremities of the arch. On the branchial filaments, the distal region of primary lamellae abruptly turn wider. The secondary, or respiratory, lamellae are reduced in size. Several mucous and chloride cells (Figs. 53 & 54) were found along the secondary lamellae, mainly on the distal portion of these filaments (Fig. 54). The epithelial cells were covered both the primary and secondary lamellae, the chloride and mucous cells were distributed primarily at the bases of the secondary lamellae (Fig. 53).

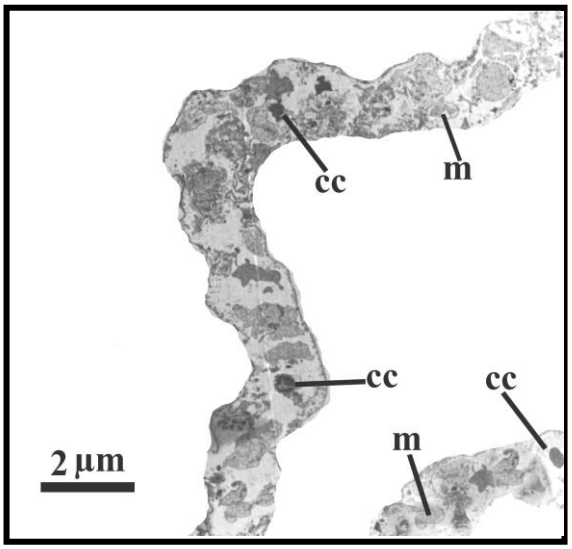


Figure (53): Transmission electron micrograph of the secondary gill lamellae of a non-infested *Mugil cephalus*. Note, the chloride cells (cc) and mucous cells (m). (X 500).

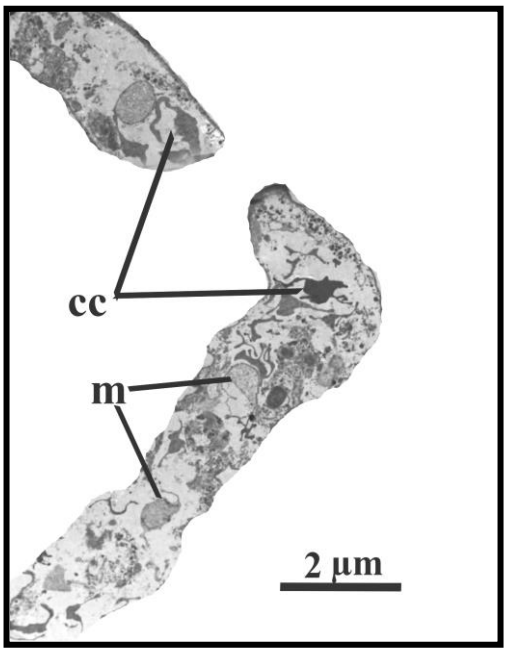


Figure (54): Transmission electron micrograph of the distal part of the secondary gill lamellae of a non-infested *Mugil cephalus*. Note, the chloride cells (cc) and well developed mucous cells (m). (X 500).

Pathological changes associated with *Lernanthropus kroyeri* infections.

i- Light microscopy studies

Lernanthropus kroyeri were primarily found attached to the ventral surface of the gill filaments, tight to the interbranchial septum (Fig. 55). Attachment involved use of the parasite's antennae to grasp the gill filaments (Fig. 56). Attachment was characterised by the antennae directed in a forward position toward the gill arch (Fig. 56). This allowed the parasite's body and egg strings (when present) to lie parallel to the filaments (Figs. 57 & 58). Attachment usually involved the antennae embracing two gill filaments (Figs. 55 & 56). This resulted in the ventral surface of the parasite hanging within the space between adjacent filaments rather than directly along the gill surface (Fig. 55). Insertion of the antennae into the filament ensured firm attachment to the gills.

Epithelial hyperplasia was a frequently recorded consequence of *Lernanthropus kroyeri* infection. In the microscopic examination, individual of *L. kroyeri* were observed in the gill filaments of fish hosts. Primary lamellae were destructed where *L. kroyeri* was penetrated to the gills. There were also lamellary edema, fusion of the secondary lamellae due to significant epithelial proliferation, mucus cell proliferation, erosion of the branchial lamellar epithelium and necrosis in tips of the primary lamellae where the parasites penetrate (Figs. 55, 57, 58 & 59). Adult female copepods were attached to the flat, lamellae bearing sides of the primary lamellae (gill filaments) by their second antennae. The body of parasite was positioned between the hemibranchs, attached to the internal face, with their axis parallel to the primary lamellae axis and with their cephalic extremities oriented towards the gill arch (Fig. 56).

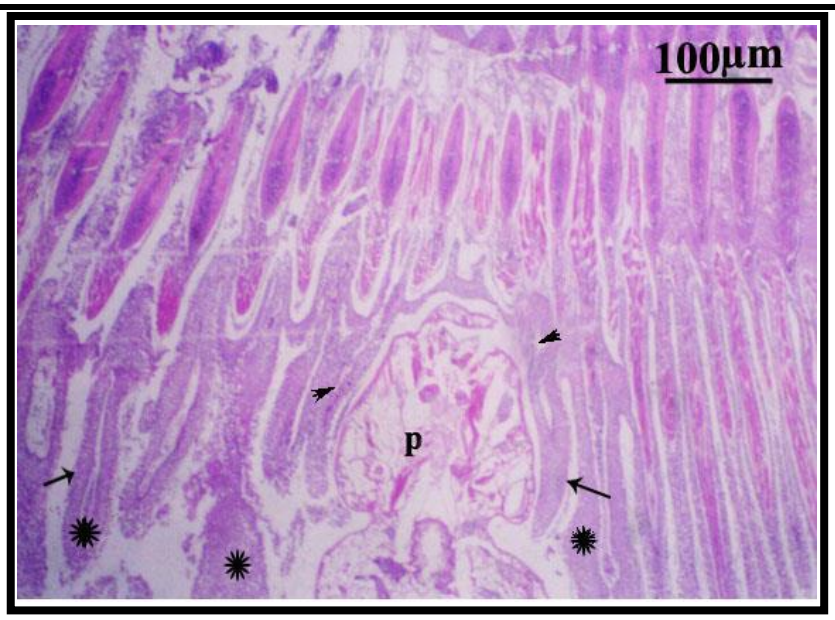


Figure (55): Photomicrograph of a paraffin wax section, stained with haematoxylin and eosin, showing *L. kroyeri* (p) attached between adjacent filaments of sea bass gill. Pressure exerted by attachment caused noticeable indentation and thinning of the filament (arrowheads), fusion of the secondary lamellae (arrows) and hyperplasia (asterisks). The body of the parasite lies between filaments.

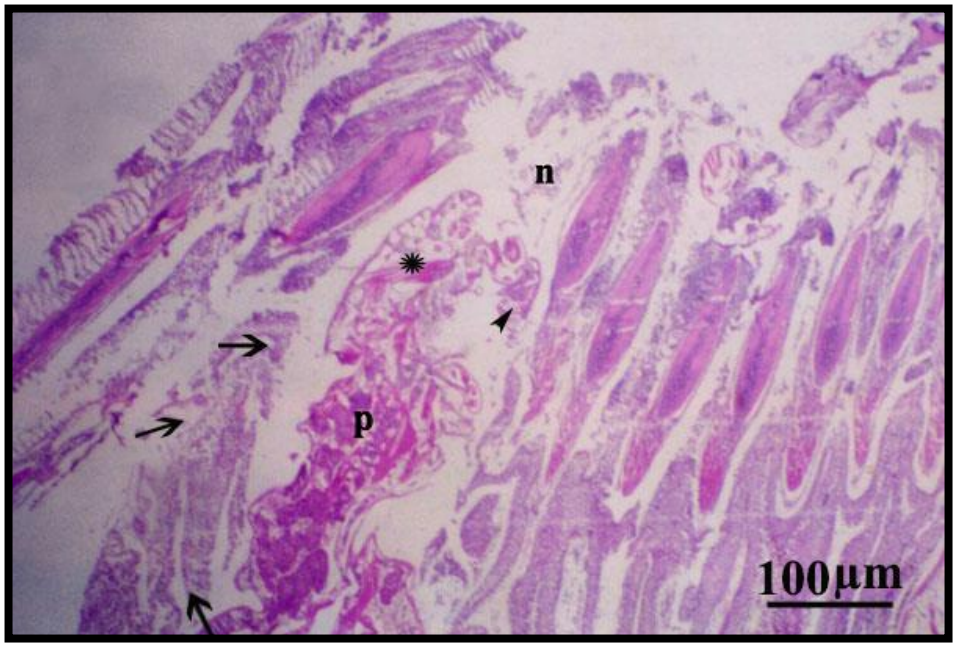


Figure (56): Photomicrograph of a paraffin wax section, stained with haematoxylin and eosin, showing *L. kroyeri* (p) attached between adjacent filaments of sea bass gill. Pathological changes at the site of attachment, included necrosis (n) and epithelial erosion, compression and haemorrhage (arrows). cephalic extremity (*); 2nd antenna (arrowhead). These were considered the combined effect of parasite attachment and feeding behaviour.

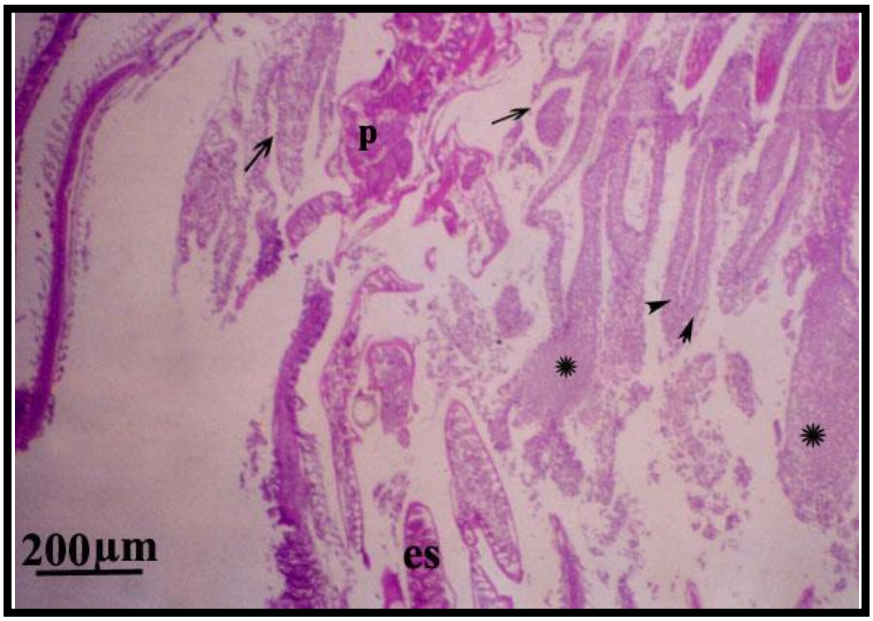


Figure (57): Photomicrograph of a paraffin wax section, stained with haematoxylin and eosin, showing attachment of *L. kroyeri* (p) to distal regions of sea bass gill filaments. Note, more pronounced damage, including hyperplastic area in the primary lamellae (arrow heads); cell proliferation (*) and loss of normal gill structure in regions between the filament (arrows). Egg sac (es).

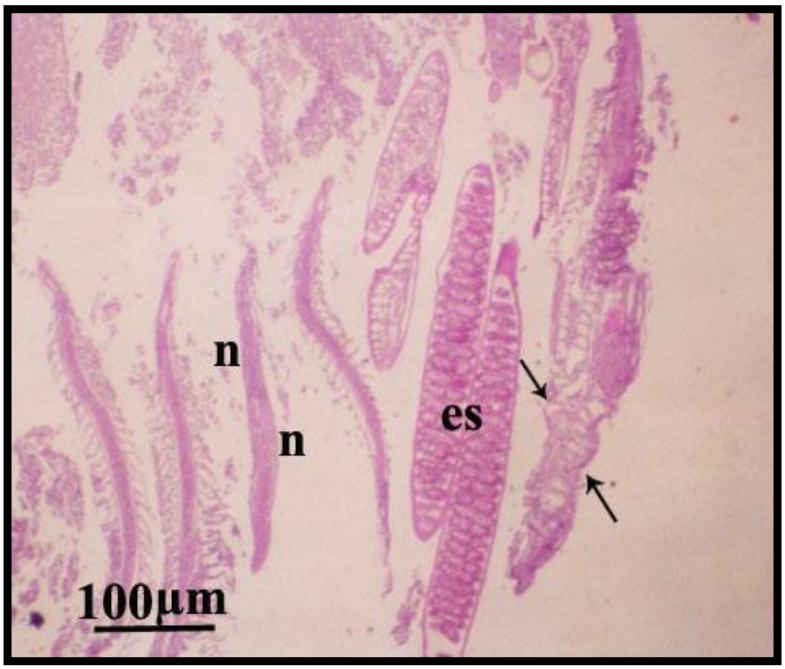


Figure (58): Photomicrograph of a paraffin wax section, stained with haematoxylin and eosin, showing longitudinal section through sea bass gill at distal region of filaments showing egg sacs (es) of *L. kroyeri* near contact the gill filament. Note, fusion of the secondary lamellae (arrows) and necrosis (n).

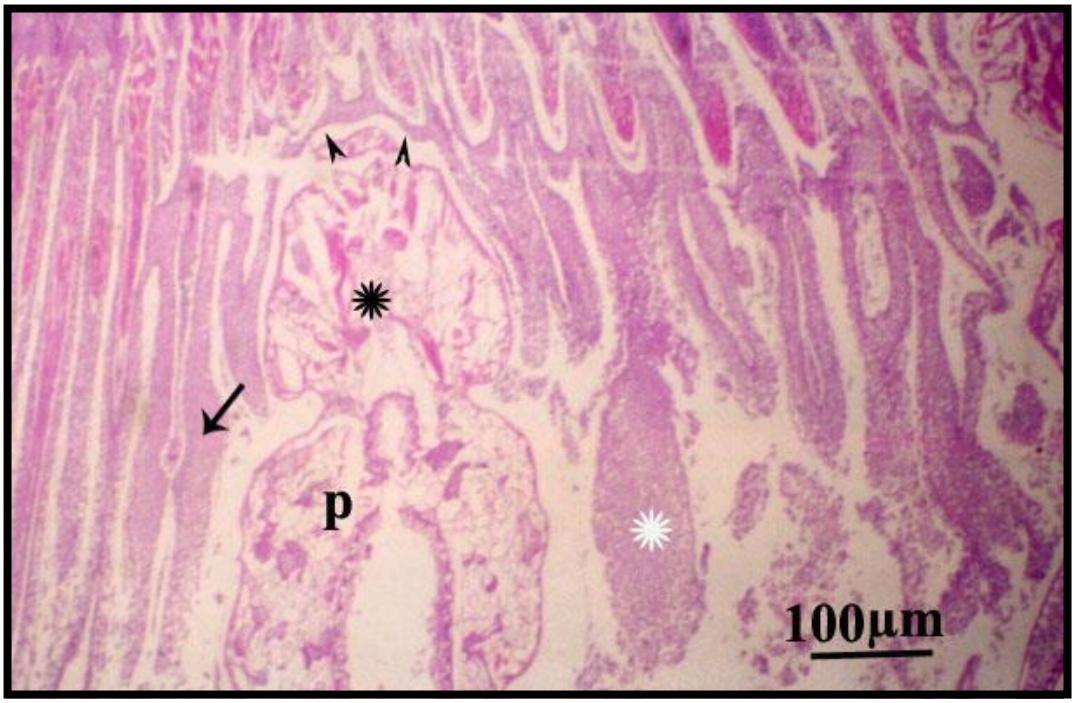


Figure (59): Photomicrograph of a paraffin wax section, stained with haematoxylin and eosin, showing *L. kroyeri* (p) attached between adjacent filaments of sea bass gill. Note, compression exerted by the cephalic extremity (*) of parasite resulting erosion of the branchial lamellar epithelium (arrowheads), hyperplasia (white stars) and noticeable indentation and thinning of the filament (arrow).

Histologically, at the site of parasite attachment, regressive phenomena prevailed: complete superficial tissue erosion with exposure of the primary lamellae cartilage, exposure of blood vessels and hyperplasia resulting from the grasping action of the 2nd antennae and maxillipeds (Figs. 55 & 59). Primary lamellae erosions, necrosis, epithelial erosion, compression and haemorrhage resulted from parasitic infection (Fig. 56). During most infections, pathological changes were localised, leaving the majority of the gill filament relatively normal (Fig. 56).

Due to the strict site specificity shown by *Lernanthropus kroyeri* , heavy parasite infections resulted in loss of space adjacent to the interbranchial septum (Figs. 55 & 59). The presence of the parasite's body within this region resulted in mechanical compression and distortion of the ventral filament surface (Fig. 59). However, this varied considerably depending upon space availability and the position of parasites within this region. The pressure exerted by the body of the parasite, combined with the gripping action of the antennae caused compression of epithelium and thinning of the filament (Fig. 59).

Many of the pathological changes associated with *Lernanthropus kroyeri* resulted from attachment, namely insertion of the antennae into the gill and the pressure exerted by the body of the parasite. The severity of pressure changes varied according to host size, space available between the hemibranchs and parasite morphology. Space was generally most limited in the region closest to the interbranchial septum. However, this region was primarily utilised by the narrow, forwardly positioned antennae (Figs. 55 & 59) which were relatively easily accommodated. The greatest pressure changes were associated with the thickest part of the parasites body, namely the anterior region of the cephalothorax (Figs. 55 & 56).

However, with increasing space availability away from the gill septum, damage remained relatively mild within this region (Fig. 56). Despite their relatively large size, the egg strings of *Lernanthropus kroyeri* were associated with few pathological changes. Space within this region minimized host parasite contact, leading to minimal gill damage (Figs. 57 & 58). This was consistent in fish hosts, where the egg strings extended beyond the gill in to the space of the opercular chamber.

In most infections, the anterior regions of *Lernanthropus kroyeri* maintained close contact with host tissues, whilst the posterior regions of the body, including 3rd and 4th thoracic legs caused little damage (Figs. 56, 57 & 58). Exceptions to this included minor flattening of the secondary lamellae and mild hyperplasia.

In semi-thin sections stained with toluidine blue, erosion, desquamation, necrosis and interruption of infected gill tissues and number of mucous cells were observed in (Fig. 60). Comparable with uninfected secondary lamellae that was seen in (Fig. 44), pathological changes included cell proliferation and necrosis of the secondary lamellae were observed (Fig. 60**)**. In addition, mucous secretion were found cover epithelial surface of the primary and secondary gill lamellae (Fig. 60).

Histopathological changes at distal region of filaments showed proliferation of gill epithelium that chronic and up to four mucous cell layers found on the surface of epithelium, epitheliocystis-like inclusions and massive loss of filament tissues (Fig. 61).

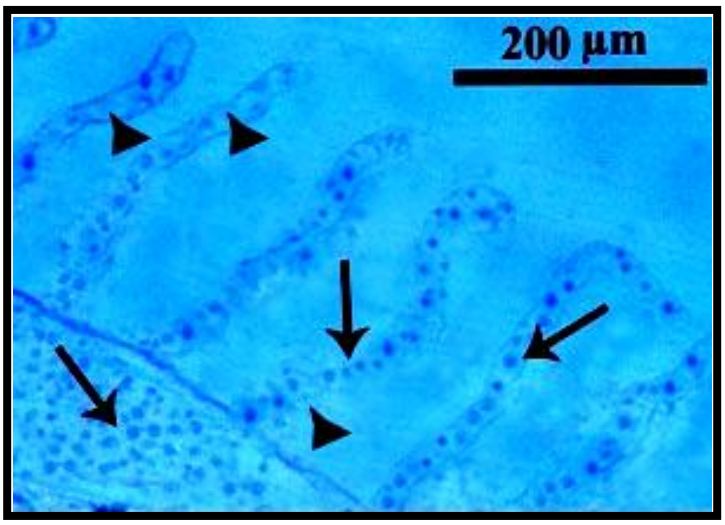


Figure (60): Photomicrograph of a semi-thin, toluidine blue-stained section showing pathological changes in infested sea bass gill including interruption and necrosis of secondary lamellae (arrowheads) and a higher numbers of mucous cells (arrows).

Note, parasite dropped from the gill filament during specimen processing.

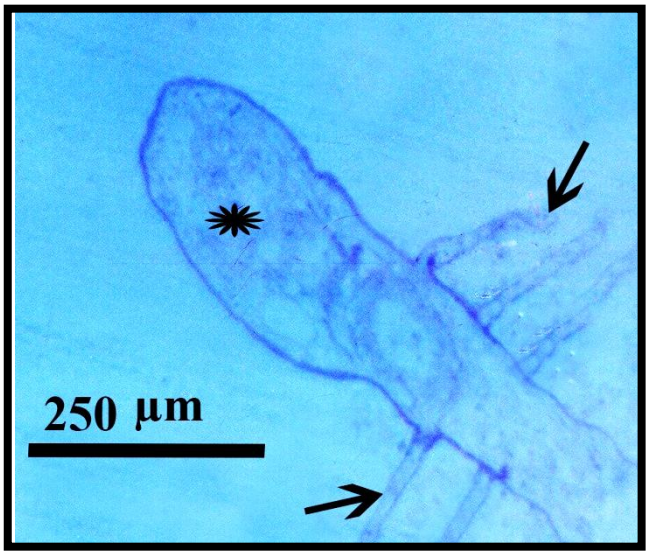


Figure (61): Photomicrograph of a semi-thin, toluidine blue-stained section showing infested sea bass gill. Note, proliferation of gill filament (*) and loss of normal gill structure (arrows).

Note, parasite dropped from the gill filament during specimen processing.

ii. Scanning electron microscopy

Scanning electron microscopy (SEM) observations revealed important information on the mode of *Lernanthropus kroyeri* attachment. Specifically, parasites anchored themselves to the primary lamellae through the piercing action of their second antennae (Figs. 62 & 63). The security of attachment is reinforced through the action of the 2nd antennae, maxillipeds, 3rd thoracic legs and 4th thoracic legs (Fig. 62). The most characteristic histopathological effect of *Lernanthropus kroyeri* resulting from clasping action of the previous appendages on the lamella, cause erosion and disruption of the tissue (Figs. 62 & 63). Fusion of the secondary lamellae was also observed in close proximity to the site of attachment (Fig. 62). At the site of parasite attachment, regressive phenomena prevailed: superficial tissue erosion with exposure of the primary lamellae cartilage, resulting from the grasping action of the 2nd antennae, 2nd maxillae and the maxillipeds (Fig. 62). Hyperplasia of the interlamellar epithelium and partial fusion of the secondary lamellae was also observed in close proximity to the site of attachment (Fig. 62). A lesion associated with female *Lernanthropus kroyeri* was compatible with depression, destruction and massive loss of filament tissues (Fig. 62). Mucous secretion were found cover epithelial surface of the primary gill lamellae (Fig. 63).

iii. Transmission electron microscopy

Ultrastructure observations revealed that cell proliferation and necrosis of the secondary lamellae (Fig. 64) of *Dicentrarchus labrax* gill filaments. Typically these growths were most pronounced about the anterior of the copepod's cephalothorax and especially just anterior to the frontal plates. A short distance from attached female *Lernanthropus kroyeri*, the surface of the interbranchial

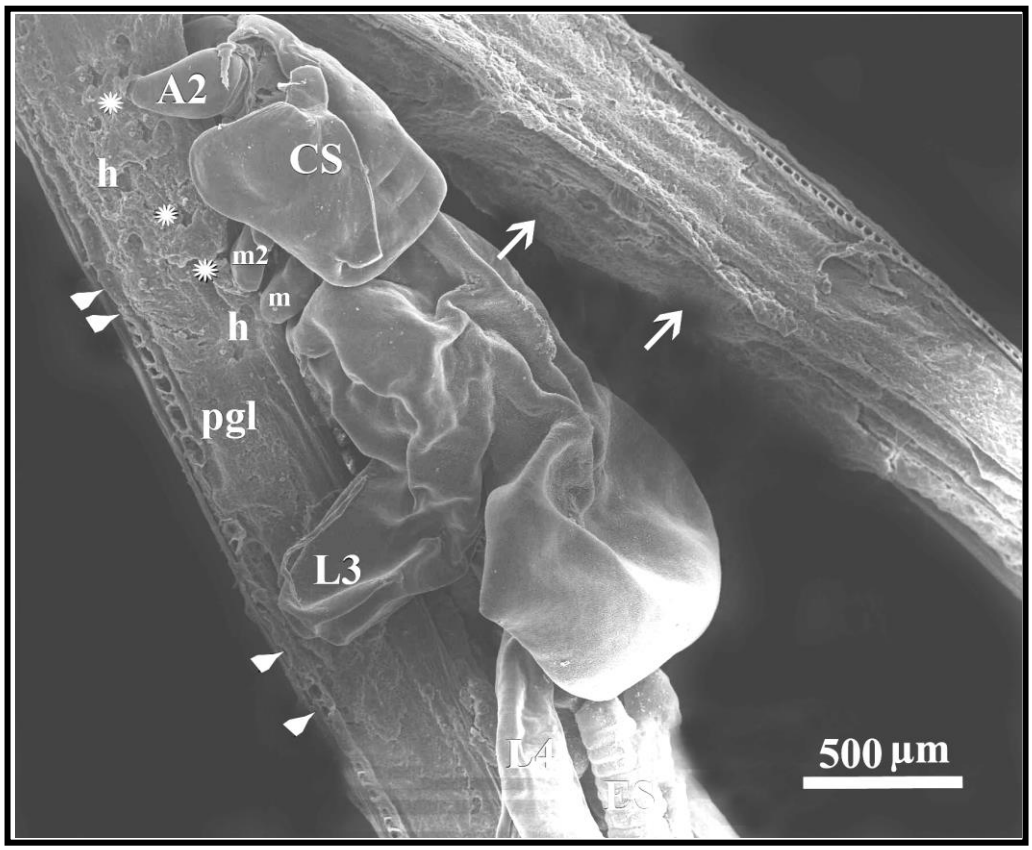


Figure (62): Scanning electron micrograph showing the attachment of adult female *Lernanthropus kroyeri* at the primary gill filament (pgl) with the 2nd antennae (A2), 2nd maxillae (m2), maxillipeds (m), 3rd legs (L3) and L4, 4th leg. This type of attachment results more pronounced damage, including hyperplasia (h), fusion of the secondary lamellae (arrowheads), erosion and disruption of the tissue (*) and depression massive loss of filament tissues (arrows). Presence of the parasite, with egg sac (ES), often resulted in displacement of adjacent gill filaments. Note, cephalic shield (CS) is directed to the base of gill filament.

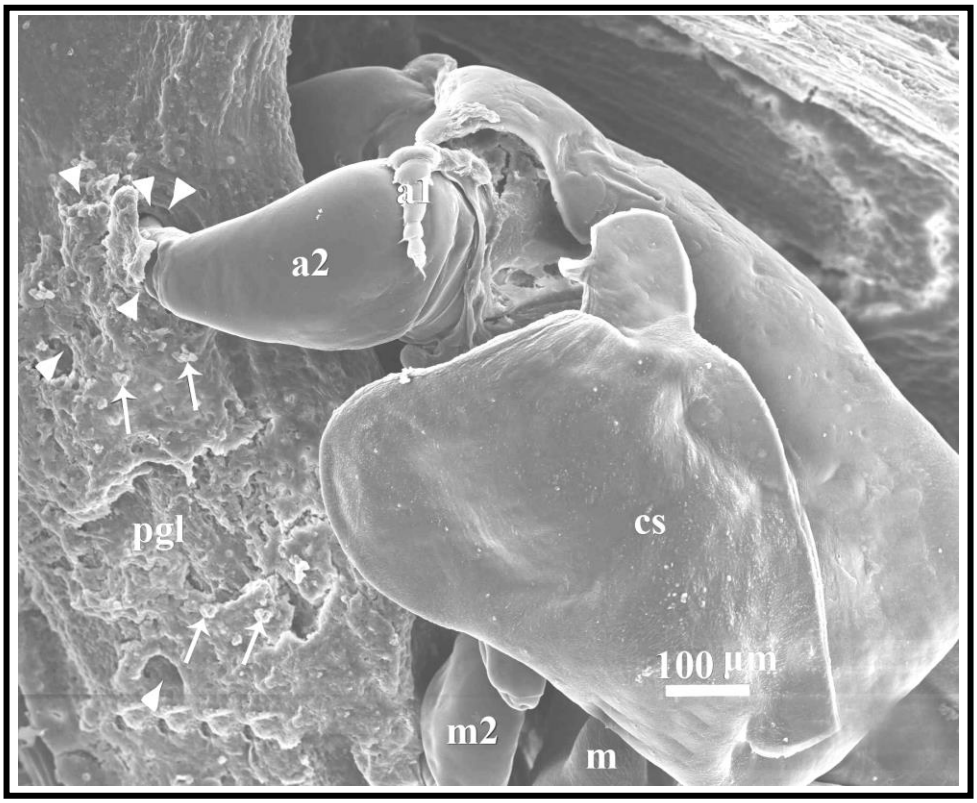


Figure (63): Scanning electron micrograph showing insertion of 2nd antennae (a2) of *Lernanthropus kroyeri* into the gill filament of sea bass caused localized erosion and disruption (arrowheads) and mucous secretion cover epithelial surface (arrows) of the primary gill filament (pgl). a1, 1st antenna; cs, cephalic shield; m, maxilliped and m2, 2nd maxilla.

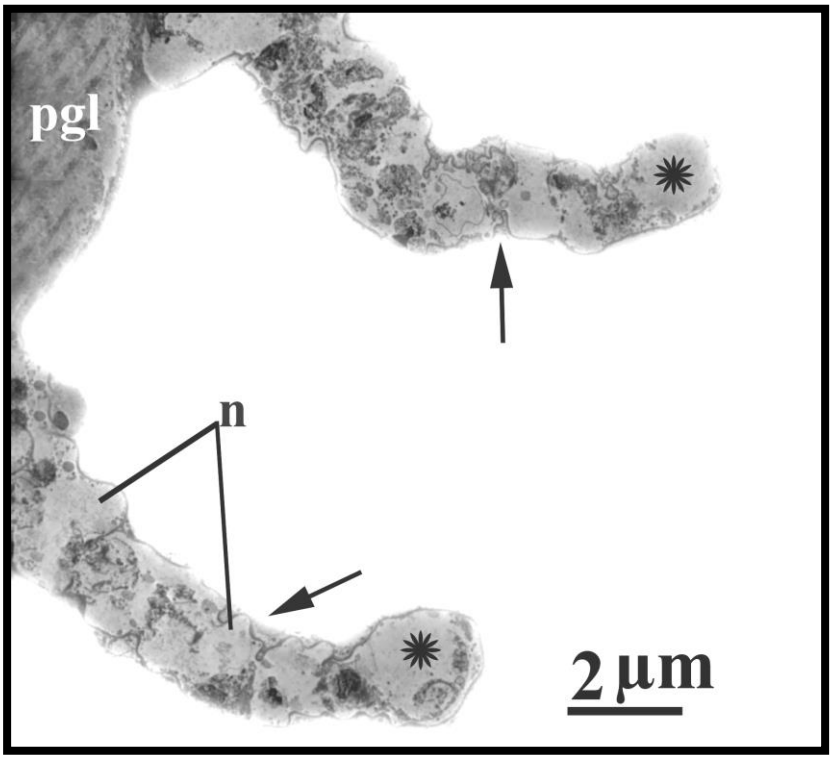


Figure (64): Transmission electron micrograph showing a part of primary gill filament (pgl) of infested gill and two secondary lamellae (arrows). Pathological changes included cell proliferation (black asterisks) and necrosis (n) of the secondary lamellae comparable with uninfected secondary lamellae. (X 500).

Note, parasite dropped from the gill filament during specimen processing.

septum appeared smooth and normal (Fig. 65). Therefore, attached female *Lernanthropus kroyeri* were either not associated with any gross epithelial lesions or attached within shallow ulcerations.

The papillomatous growths associated with the attachment of female *Lernanthropus kroyeri* were characterized by marked epithelial hyperplasia with disorganization of epithelial layers, necrosis and massive loss of filament tissues (Fig. 65).

Observations with the TEM have confirmed that adult female *Lernanthropus kroyeri* attached to primary lamellae (gill filaments) with their claw-like second antennae close to the gill arch near the base of filaments (Fig. 66). The parasite's body lies between the hemibranchs with the axis parallel to the primary lamellae axis and with its cephalic extremity oriented towards the gill arch.

Through the piercing action by means of its cephalothoracic appendages especially, the second antennae, these deep penetrations of this appendage into the gill tissue produce major histopathological changes include degeneration, enhanced mucous production, congestion, necrosis, massive loss of filament tissues haemorrhages and primary lamellae erosions were encountered (Fig. 66).

TEM has revealed that female *Lernanthropus kroyeri* was found to induce histopathological changes at distal region of filaments showing proliferation of gill epithelium that chronic and up to four mucous cell layers found on the surface of epithelium, epitheliocystis-like inclusions and massive loss of filament tissues (Fig. 67). Moreover, massive loss of filament tissues and necrosis were observed (Fig. 68). Erosion, desquamation, necrosis and degeneration of infected gill tissues and number of mucous cells were occurred at the TEM level (Fig. 69).

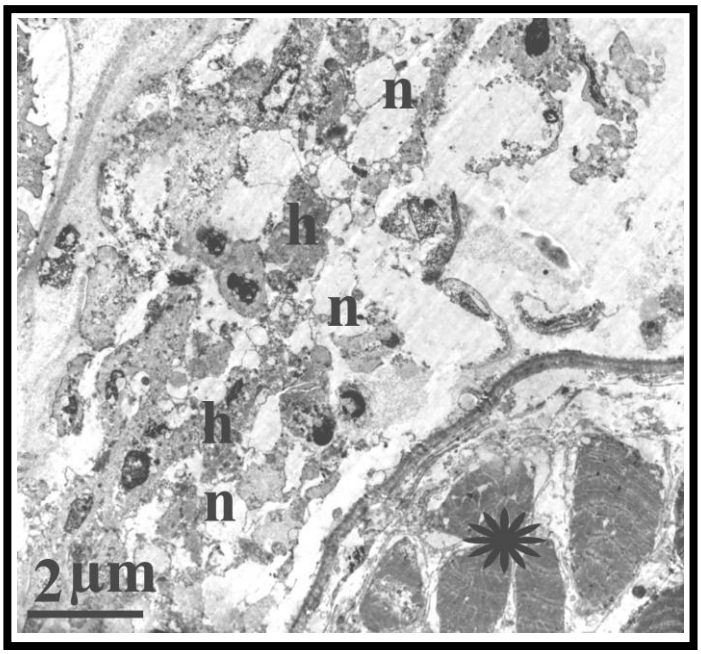


Figure (65): Transmission electron micrograph showing a section through the anterior end of female *Lernanthropus kroyeri* (*) at the site of attachment causing hyperplasia (h) and necrosis (n) of the host tissues. (X 500).

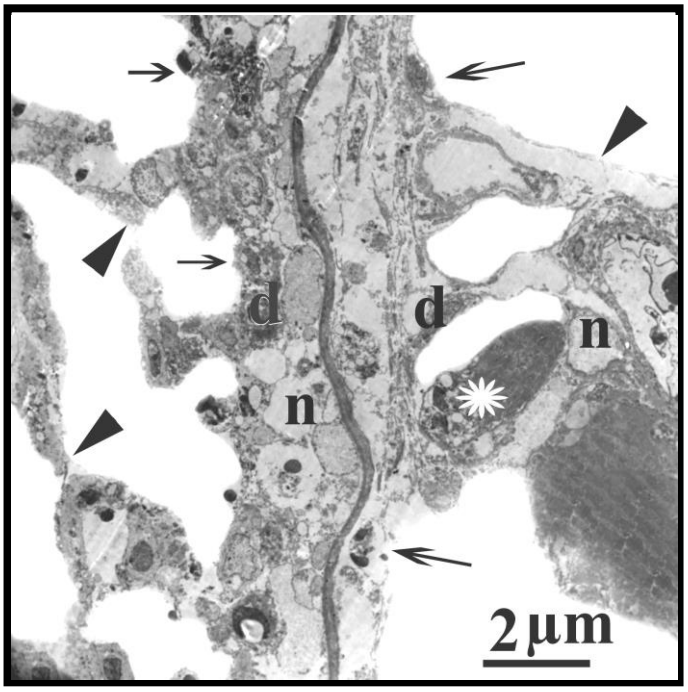


Figure (66): Transmission electron micrograph showing the parasite attached by clawed second antenna (asterisk) deeply penetrating gill tissue caused fusion of the secondary lamellae (arrowheads), loss of normal gill structure and primary lamellae erosions (arrows), degeneration (d) and necrosis (n). (X 500).

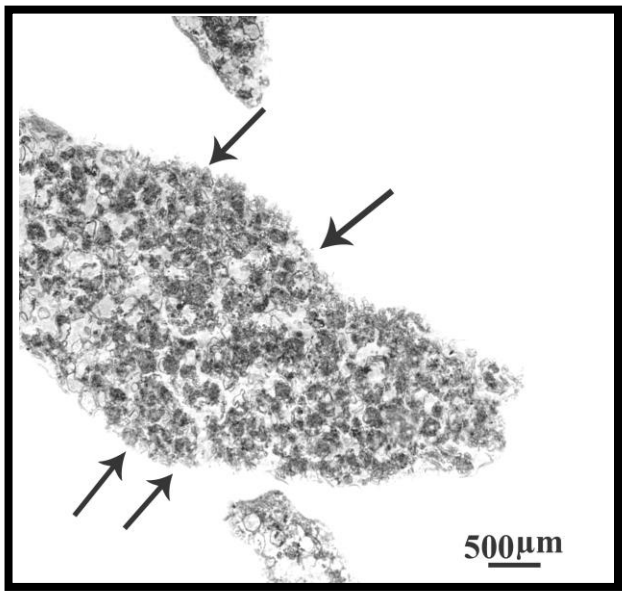


Figure (67): Transmission electron micrograph showing longitudinal section through infested sea bass gill at distal region of filaments. Note, proliferation of gill epithelium, epitheliocystis-like inclusions and massive loss of filament tissues (arrows). (X 1500).
Note, parasite dropped from the gill filament during specimen processing.

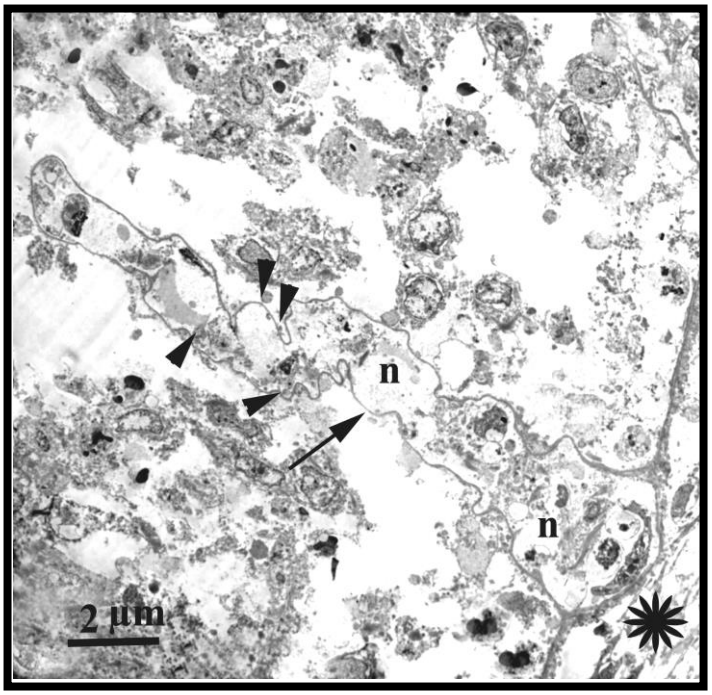


Figure (68): Transmission electron micrograph showing erosion and destruction (arrowheads) of secondary lamella (arrow) of infested gill, loss of normal gill structure (*) and necrosis (n). (X 500).
Note, parasite dropped from the gill filament during specimen processing.

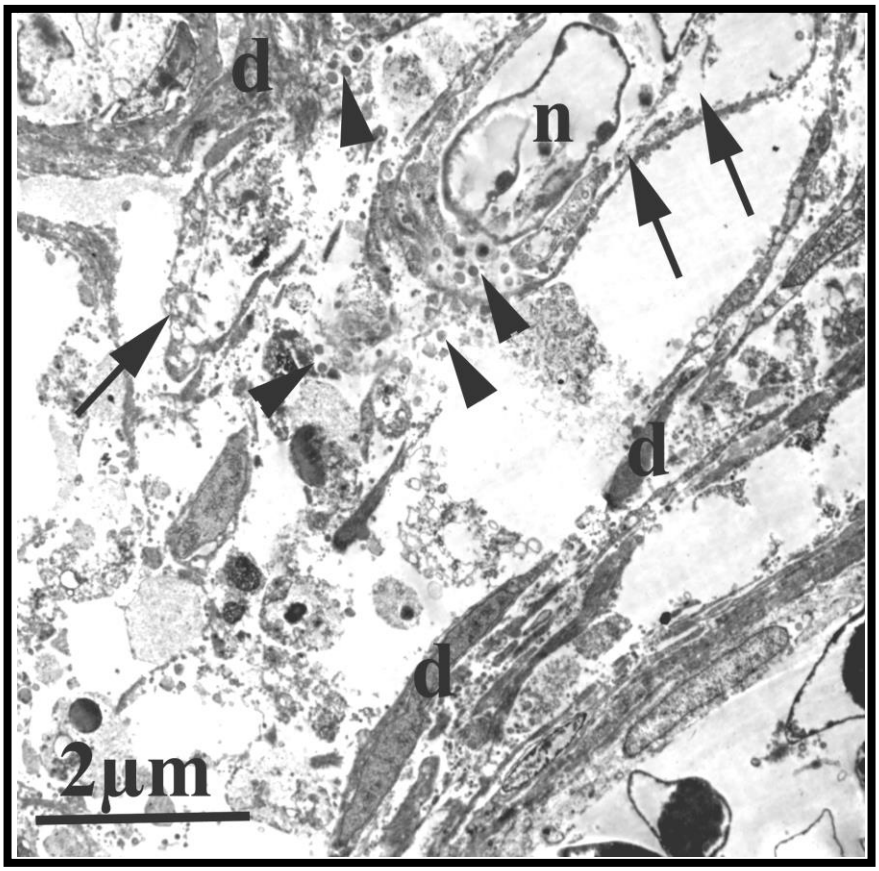


Figure (69): Transmission electron micrograph showing erosion, desquamation (arrows), necrosis (n), degeneration (d) of infested gill tissues and number of mucous cells (arrowheads) was observed. (X 1000).

Note, parasite dropped from the gill filament during specimen processing.

Pathological changes associated with *Ergasilus versicolor* infections.

i- Light microscopy studies

At the light microscope level, female *Ergasilus versicolor* was found to induce histopathological changes at the site of attachment of the gill lamellae of *Mugil cephalus*. The attachment usually involved the 2nd antennae grasping two gill filaments. In most infections, the anterior regions of *Ergasilus versicolor* maintained close contact with host tissues, whilst the posterior regions of the body, including swimming thoracic legs and urosome caused little damage (Figs. 70 & 71).

An additional type of lesion was observed of *Ergasilus versicolor* consisting of epithelial hyperplasia, loss of normal gill structure and massive loss of filament tissues in gill filaments of *Mugil cephalus* lead to fusion of the secondary lamellae (Fig. 70).

Lesions associated with female *Ergasilus versicolor* appeared with necrosis of secondary gill lamellae and massive loss of filament tissues. At the light microscope level, the crustacean parasite (*Ergasilus versicolor*) exerts a compression against the gill tissue at the site of attachment to the gill lamellae of *Mugil cephalus***,** with the exception of mild distortion, epithelial compression and fusion of primary filaments (Fig. 71).

Due to the obscured position of parasites on the ventral surface of the gills, it was difficult to make direct observations of the feeding activity of *Ergasilus versicolor*. However, damage to the filaments adjacent to the mouth parts included

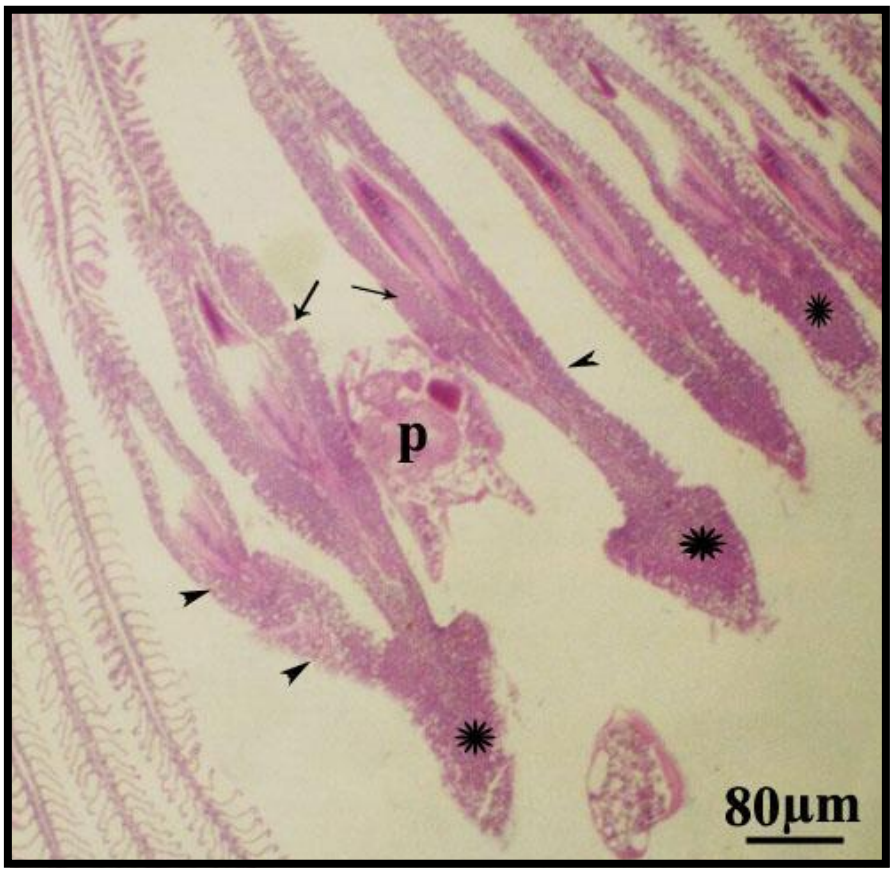


Figure (70) : Photomicrograph of a paraffin wax section, stained with haematoxylin and eosin, showing *Ergasilus versicolor* (P) attached between adjacent filaments of mullet gill. There is more pronounced damage, including hyperplasia (*), loss of normal gill structure (arrows) and fusion of the secondary lamellae (arrowheads).

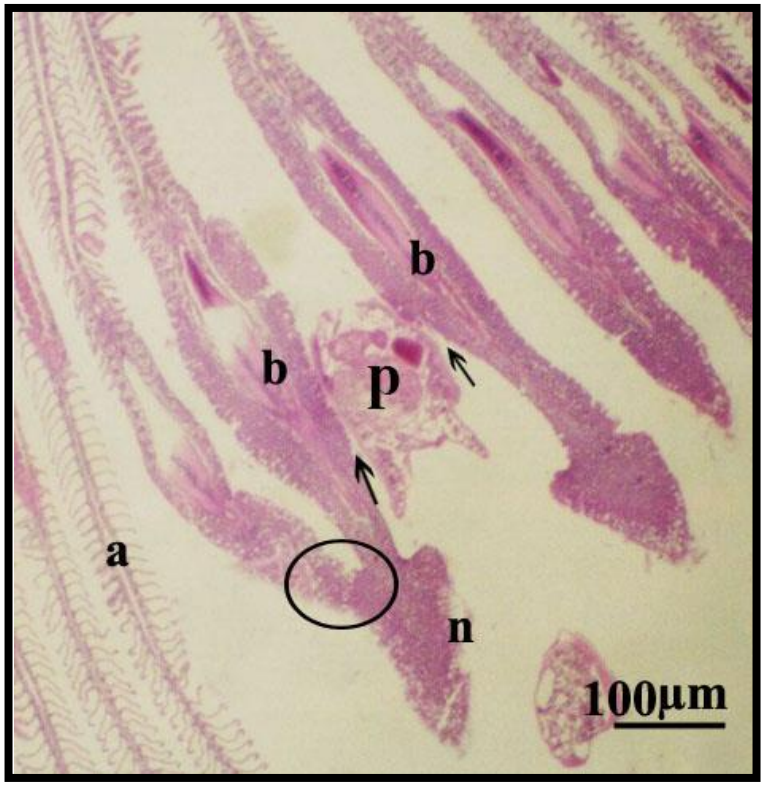


Figure (71): Photomicrograph of a paraffin wax section, stained with haematoxylin and eosin, showing a comparison of un infested (a) and infested (b) filaments of mullet gill. A single *E. versicolor* (*) is attached between adjacent filaments with the exception of mild distortion and epithelial compression (arrows), necrosis (n) and fusion of two primary filaments (circle).

erosion, loss of normal gill structure and compression of the epithelium (Figs. 70 & 71). This was often most noticeable on the lateral surfaces of the filaments, suggesting that *Ergasilus versicolor* may adjust position during feeding. The combination of attachment and potential feeding appeared to place mild distortion and epithelial compression (Fig. 71).

Lesions were observed on the gill filaments including necrosis of secondary gill lamellae, absence of gill epithelium and massive loss of filament tissues (Fig. 72). In semi-thin sections stained with toluidine blue, histopathological changes at distal region of filaments showed proliferation of gill epithelium (Fig. 72). The most characteristic histopathological effect of *Ergasilus versicolor* were erosion, disruption of the tissue and loss of normal gill structure (Fig. 73). Destruction and fusion of the secondary lamellae was also observed (Fig. 73).

ii-Scanning electron microscopy

The major features of female *Ergasilus versicolor* infections on the gill filaments of the fish studied include destruction and erosion of the gill filaments and lamellae exerted by the force of attachment and feeding of the parasite (Fig. 74) and the resultant hypertrophy of the underlying epithelia reducing the surface area for effective respiration. This could lead to reduced growth and secondary infection affecting the survival of fish. Mucus secretion were found cover epithelial surface of the primary gill lamellae (Fig. 74).

Attachment involved use of the parasite's antennae to grasp the gill filaments. This brought the mouth-parts located on the underside of the body into close contact with the gill tissue (Fig. 74). Attachment was characterised by the antennae directed in a forward position toward the gill arch (Figs. 74, 75 & 76).

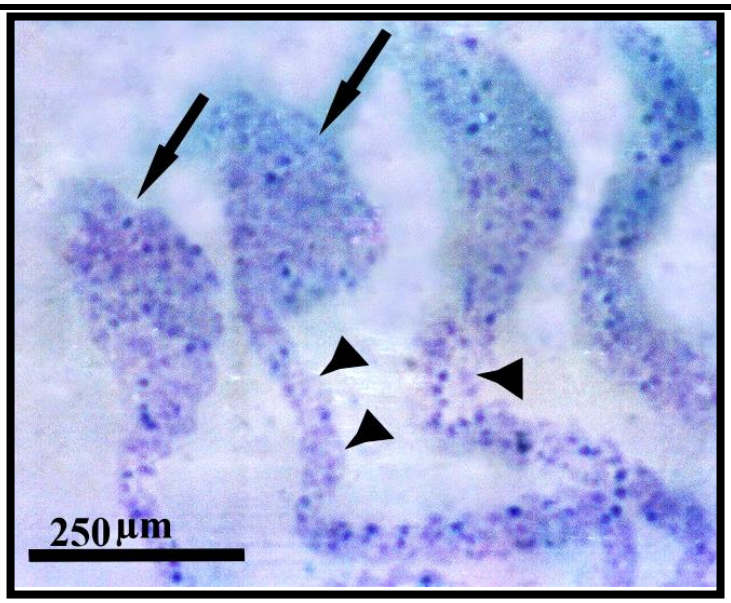


Figure (72): Photomicrograph of a semi-thin, toluidine blue-stained section showing infested gill of mullet. Pathological changes included cell proliferation (arrows) and absence of gill epithelium (arrowheads) of the secondary lamellae comparing with uninfected secondary lamellae.

Note, parasite dropped from the gill filament during specimen processing.

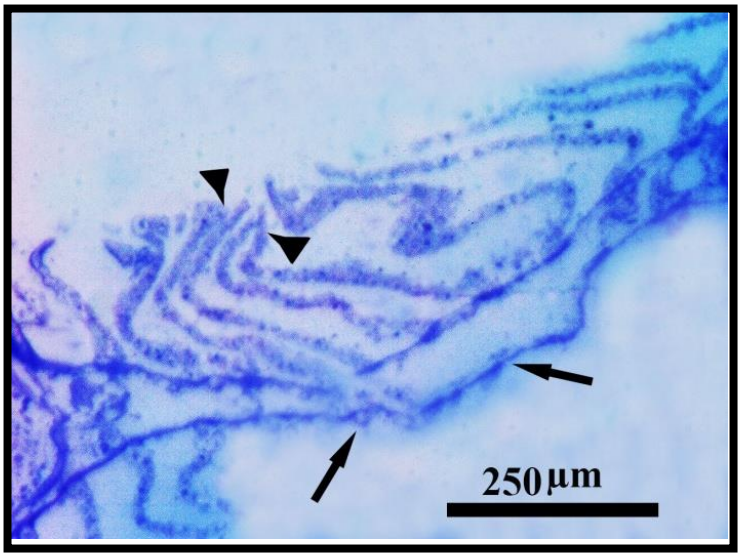


Figure (73): Photomicrograph of a semi-thin, toluidine blue-stained section showing infested gill of mullet. Note, loss of normal gill structure and primary lamellae erosions (arrows) and fusion of secondary gill lamellae (arrowheads).

Note, parasite dropped from the gill filament during specimen processing.

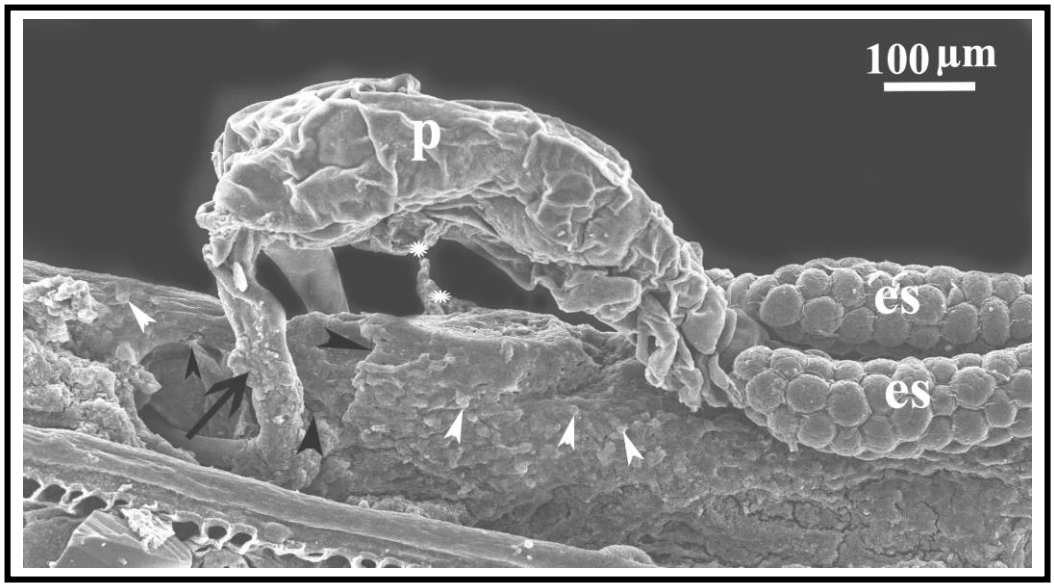


Figure (74): Attachment of female *Ergasilus versicolor* (p) with egg sacs (black stars), showing use of antennae (arrow) to grip gill filaments causing destruction and erosion of the gill filaments and lamellae (black arrowheads) and mucous secretion cover epithelial surface (white arrowheads). Note, the orientation of parasite body along the gill filaments. This allowed the mouth-parts (white star) to come into close contact with the gill surface.

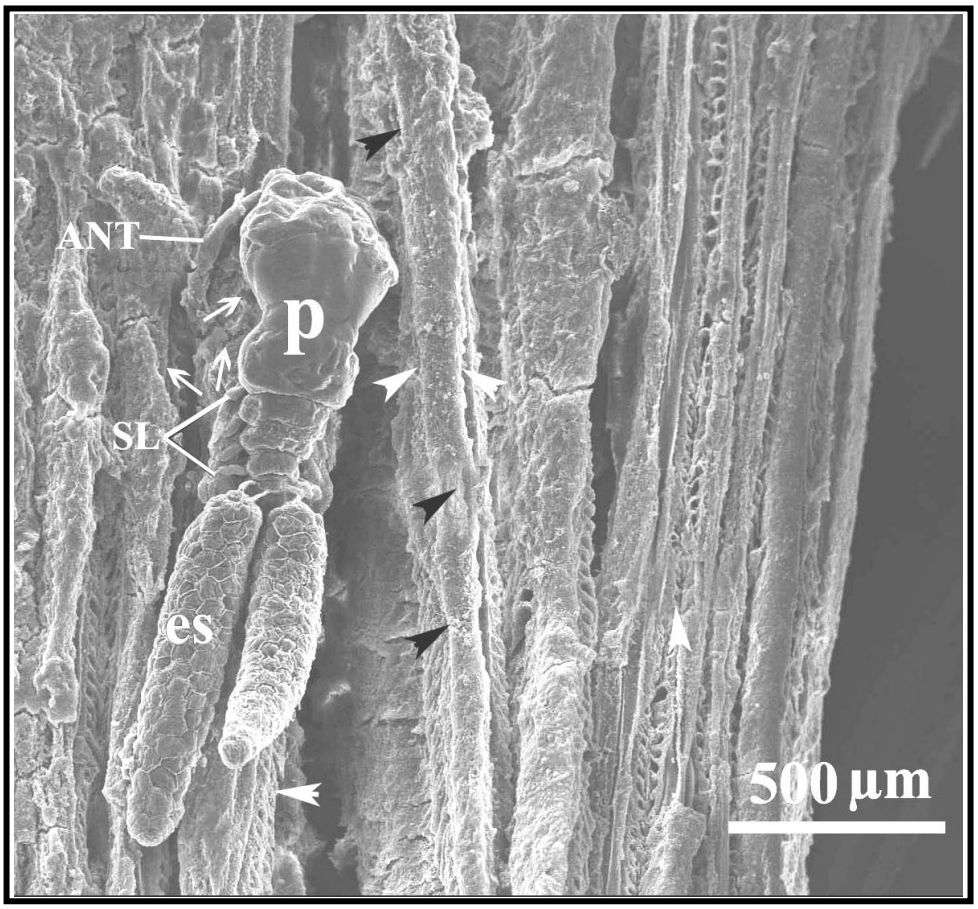


Figure (75): Scanning electron micrograph showing attachment of adult female *Ergasilus versicolor* (P) at the gill filament with the 2nd antennae (ANT) and swimming legs (SL). The parasite shows more pronounced damage, including erosion of the gill filaments (arrows), fusion of the secondary lamellae (white arrowheads), thinning of tissue with loss of normal gill structure (black arrowheads), egg sac (es).

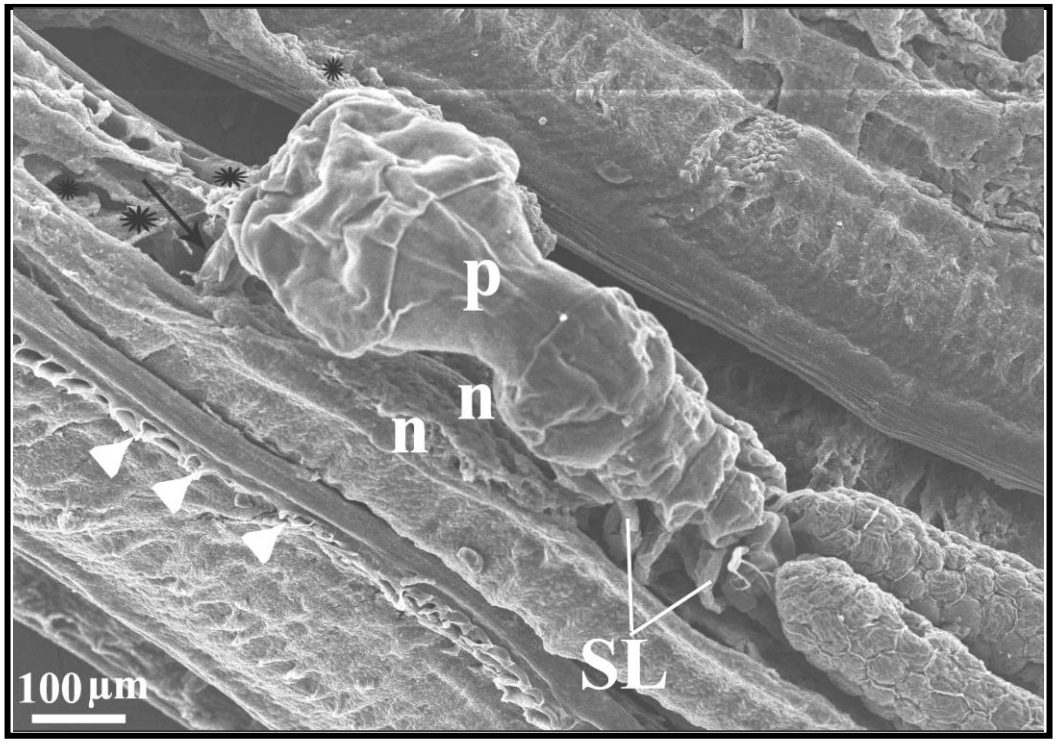


Figure (76): Scanning electron micrograph showing *Ergasilus versicolor* between adjacent gill filaments. Pathological changes was recorded as a result of parasite (P) attachment with 2nd antennae (arrow). These changes include erosion of tissue (*), relatively fusion of the secondary lamellae (arrowheads), necrosis (n) and swimming legs (SL).

This allowed the parasite's body and egg strings to lie parallel to the filaments. Insertion of the terminal segment of the antennae into the filament (Figs. 74, 75 & 76) ensured firm attachment to the gills.

Many of the pathological changes associated with *Ergasilus versicolor* resulted from attachment, namely insertion of the antennae into the gill and the resultant pressure exerted by the body of the parasite. This lead to more pronounced compression of epithelium and thinning of tissue with loss of normal gill structure (Fig. 75). These changes were occasionally accompanied by localised necrosis of the epithelium (Fig. 76).

In most infections, the anterior regions of *Ergasilus versicolor* maintained close contact with host tissues, whilst the posterior regions of the body, including the swimming legs and urosome caused little damage (Figs. 75 & 76).

Due to the obscured position of parasites on the ventral surface of the gills, it was difficult to make direct observations of the feeding activity of *E. versicolor*. However, damage to the filaments adjacent to the mouth parts included erosion, fusion of secondary lamellae and compression of the epithelium (Figs. 75 & 76).

iii-Transmission electron microscopy

The ultrastructure studies have revealed enhanced mucous production, congestion, haemorrhages and secondary lamellae erosions in infested gills by the copepod parasite *Ergasilus versicolor*. Erosion, desquamation and necrosis of secondary lamellae of *Mugil cephalus* were observed also(Figs. 77, 78 & 79). At the distal part of the infested lamellae, proliferation of the interlamellar epithelium

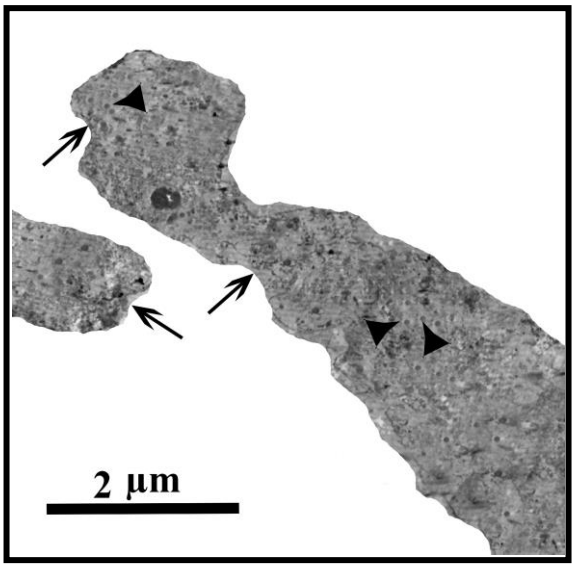


Figure (77): Transmission electron micrograph showing proliferation of secondary lamellae of *Mugil cephalus*, mucous production (arrowheads) and erosion of gill lamellae (arrows). (X 1000).

Note, parasite dropped from the gill filament during specimen processing.

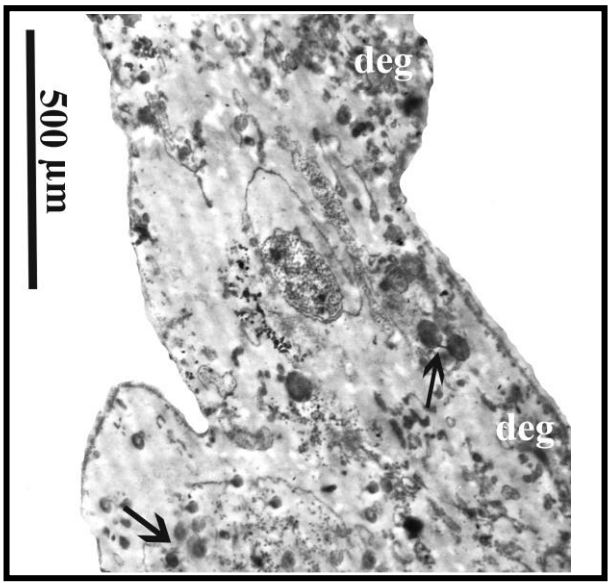


Figure (78): Transmission electron micrograph showing a part of secondary gill lamella of infested *Mugil cephalus*, pathological changes included mucous production (arrows) and degeneration of gill lamellae (deg). (X 4000).

Note, parasite dropped from the gill filament during specimen processing.

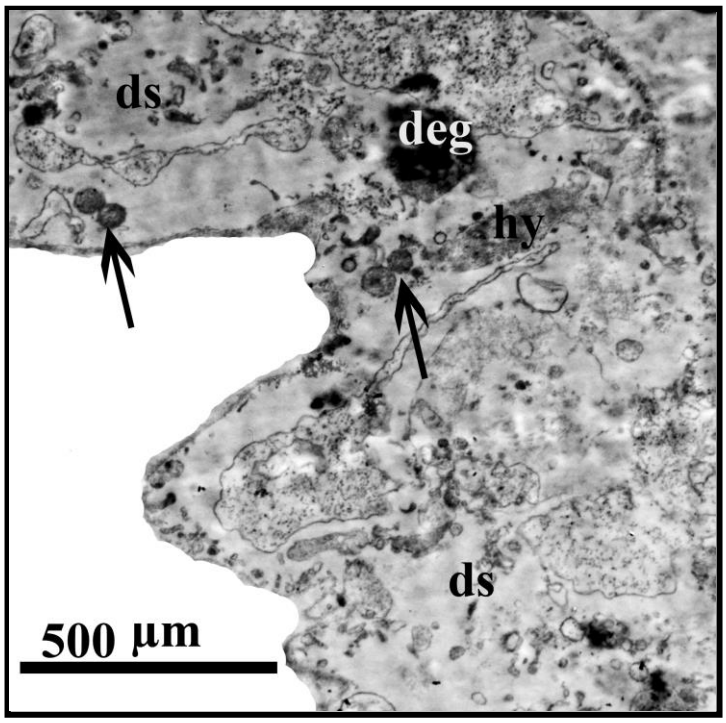


Figure (79): Transmission electron micrograph showing desquamation (ds), degeneration (deg), mucous production (arrows) and hyperplasia (hy) of infested gill tissues of *Mugil cephalus*. (X 4000).

Note, parasite dropped from the gill filament during specimen processing.

induced lamellar fusion were reported. Moreover, massive mucous cell proliferation were observed in the secondary lamellae (Fig. 77).

At the transmission electron microscope level, histopathological changes of the gill lamellae of *Mugil cephalus* included desquamation, degeneration, mucous production and hyperplasia of infected gill tissues (Fig. 79). Lesions associated with female *Ergasilus versicolor* were compatible with necrosis of secondary gill lamellae, degeneration of gill lamellae and massive loss of filament tissues (Fig. 78).

Discussion

Gills are the major respiratory organs in fishes and all metabolic pathways depend upon the efficiency of the gills for their energy supply and damage to these vital organs cause a chain of destructive events, which ultimately lead to respiratory distress. Pronounced secretion of mucus over the gill lamellae has been observed during malathion stress. Secretion of mucus over the gill curtails the diffusion of oxygen **(David *et al.*, 2002)** which may ultimately reduce the oxygen uptake by the animal.

The SEM is a technique that allows the study of the damage of surface ultrastructure of the gill epithelium that cannot be revealed by light or TEM **(Devos *et al.*, 1998).** In contrast the present study showed that the gills of *Dicentrarchus labrax* and *Mugil cephalus* infested with the copepod parasites, *Lernanthropus kroyeri* and *Ergasilus versicolor* presented a higher occurrence of histopathological lesions such as hypertrophy, fusion of secondary lamellae, edema and mucus openings. The damages observed in the gills may cause a decrease in free gas exchange, and may affect the general health of fish (**Tovell and Skidmore, 1972**).

Lernanthropus is known to cause some pathological effects on its host. **Manera and Dezfuli (2003)** reported that erosion, desquamation and necrosis of the secondary lamellae near the site of copepod *Lernanthropus kroyeri* attachment and the terminal claw of the second antennae lacerated tissue and vessels of infected gill. **Bahri *et al.*, (2002)** used *Lernanthropus kroyeri* as a bioindicator of *Dicentrarchus labrax* and *Dicentrarchus punctatus* in Tunisian Inshore Areas.

The present study has revealed that there histopathological changes associated with the attachment and feeding habit of the copepod parasite

Lernanthropus kroyeri resulting major lesions at the site of attachment. These observations indicate localized erosion, disruption and mucous secretion cover epithelial surface of the primary gill filament and lamellae erosions on gills determined from parasitic infection. Also, the present study has revealed that the host response to *Lernanthropus kroyeri* is represented by hyperplasia , fusion of the secondary lamellae, erosion and disruption of the tissue and depression massive loss of filament tissues.

Histopathological changes such as haemorrhages, hyperplasia, and necrosis along the secondary lamellae of gill filaments were seen with greater severity at the site of attachment of *Lernanthropus kroyeri* **Humphrey *et al*., (2006).** Furthermore, **Roubal (1989)** reported 'compression, deformation, hyperplasia, oedema, cellular infiltration and haemorrhage' in the epithelium, and 'haemorrhage, oedema and infiltration' in the subepithelial region of the gill filaments of *Acanthopagrus australis* infected with *Lernanthropus* sp.

Similar results were observed by **Abu Samak (2005)** where the infection with females *Lernanthropus kroyeri* is accompanied by increased proliferation of primary and/or the secondary lamellar epithelium. Also, **Abu Samak (2005)** reported that both sexes specially female parasitic copepod *L. kroyeri* are very harmful for their host, cause either directly host death or abnormally slow growth, weight loss, blood flow difficulties, failure in gill functions and increase of second infection. **Kabata (1970)** listed 3 types of local effect that parasitic copepods can effect on the gills of fish: occlusion of the branchial circulation, destruction due to the pressure of feeding, and hypertrophy. It has been demonstrated that copepods can cause excessive damage to fish.

Manera and Dezfuli (2003) attributed the massive disruption of tissue and extensive haemorrhaging to the blood-feeding activity of *L. kroyeri*, it has been

demonstrated that copepods can cause excessive damage to fish. With regard to host size and ectoparasite settlement in *Dicentrarchus labrax* parasitized with *Lernanthropus kroyeri*, an increase in the intensity of infection in fish hosts. *Lernanthropus* species attach to the host gill by means of the piercing action of the antennae, which are assisted by the maxillipeds and the modified third legs **Manera and Dezfuli (2003).** According to **Davey (1980),** the female's preference for this site results from an adaptation in her respiration system allowing her to exploit the strong branchial ventilation currents in this region.

In the present study, the damage was clearly seen under scanning electron microscopy (SEM) as the hooked end of the antenna was embedded on the gill filament. Histopathological changes such as haemorrhages, hyperplasia, and necrosis along the secondary lamellae of gill filaments were observed with greater severity at the site of attachment **Beng *et al.*, (2012).** These findings were similar to the damage done by *Lernanthropus* sp. to the gills of sea bass (*D. inbrax)* reported by **Humphrey *et al*., (2006).** They reported localised destruction of gill tissue with haemorrhages at the attachment sites. *Lernanthropus kroyeri* was known to cause histo-pathological damage that facilitates secondary infection **(Manera and Dezfuli, 2003).**

Pathological changes such as necrosis in epithelial tissue and ligament, increase in mucus secretion and narrowing of capillary veins are commonly observed during attachment of copepod parasites (**Kinne, 1984).** Infestations of lernanthropids in mostly marine fish were reported to cause pathological symptoms such as desquamation and necrosis to the secondary lamellae near the site of attachment **(Jithendran *et al*., 2008).** In their reports, lernanthropids were considered as serious pathogens in many species of wild fish and cage-cultured sea bass.

Furthermore, **Beng *et al.*, (2012)** observed pathological impacts under SEM caused by *Lernanthropus sp.* as a result of attachment with the antenna, maxilla and maxilliped together and the mandible when the parasite also fed on the host tissues. Generally, fish infested with *Lernanthropus* spp. showed pathological symptoms such as respiratory distress, lethargy, dark coloured skin, increase in mucus secretion and mortality in small fish (**Henry *et al.*, 2009).**

Compared with the previous results, the present study led to conclude that the damage by *Lernanthropus kroyeri* was caused by the combined actions of the 2nd antennae, 2nd maxillae and maxillipeds together resulting in more pronounced impacts, including hyperplasia, mucus secretion, respiratory distress, failure in gill functions, fusion of the secondary lamellae and localized erosion and disruption of the primary gill filament. The pressure exerted by the body of the parasite, combined with the gripping action of the antennae caused compression of epithelium and thinning of the filament, epithelial proliferation and compression.

The gills of fish are susceptible to a wide range of disease conditions due to their direct contact with the environment and exposure to a variety of irritants, parasites and pollutants **(Eller, 1975; Smith and Piper, 1975; Karlsson, 1983; Hoole *et al.*, 2001; Ferguson *et al.*, 2006).** Parasitic copepods are among some of the most damaging parasites of fish gills **(Kabata, 1970; Paperna and Zwerner, 1982; Abdelhalim, 1990; Domitrovic, 1998; Dezfuli *et al.*, 2003).**

Due to the pathogenicity of some ergasilids, the pathology caused by their feeding and attachment has received considerable attention. Studies include observations of *Ergasilus versicolor* **Wilson, 1911.** According to **Kabata (1970)** ergasild parasites cause damage to their hosts through attachment mechanisms,

feeding behaviour, mechanical damage from pressure exerted by the body of the parasite, and reactions of the host to infection.

Present observations indicate that the most pronounced pathological changes caused by *Ergasilus versicolor* were associated with attachment. This was characterised by epithelial hyperplasia and pressure changes resulting from use of the parasites antennae. Forceful attachment, combined with the presence of the parasites body between the hemibranchs also caused filament displacement, epithelial compression, lamellar distortion, localised necrosis and hyperplasia. Pressure exerted on the ventral filament surface caused occasional compression of the efferent arteriole. Blood vessel disruption, involving occlusion and haemorrhage is a well-documented characteristic of many ergasilid infections, leading to reduced blood flow and respiratory potential **(Kabata, 1970; Roubal, 1987; Abdelhalim, 1990; Lester and Roubal, 1995).** *Ergasilus versicolor* caused pathological changes of the gill tissues of *Carpiodes cyprinus*, *leiobus cyprineffus* and *Ajoxostoma carinatum* **(Walker, 2007)**.

Attachment of *Ergasilus versicolor* usually involved contact with two gill filaments. This behaviour combined with the dorso-ventrally flattened body of the parasite may reduce resistance to water flow. Copepod parasites can alter water flow across the gills and disrupt normal branchial ventilation **(Leonardos and Trilles, 2003; Kearne, 2005).**

This attachment pressure combined with the feeding behaviour of the parasite, causes severe epithelial erosion, constriction and exposure of the branchial arterioles and fusion of lamellae along the lateral filament surface. Gill parasites, including ergasilids may increase susceptibility of hosts to viral, fungal and bacterial infections **(Ravichandran *et al.*, 2001; Busch *et al.*, 2003).**

At the light microscope level, the present study revealed that the copepod parasite (*Ergasilus versicolor*) exerts a compression against the gill tissue at the site of attachment to the gill lamellae of *Mugil cephalus***,** with the exception of mild distortion, epithelial compression and fusion of primary filaments. This lead to more pronounced epithelial hyperplasia, loss of normal gill structure and massive loss of filament tissues. The combination of attachment and potential feeding appeared to place mild distortion and epithelial compression.

The present study revealed that the response of the *Mugil cephalus* fishes infested with *Ergasilus versicolor* showed necrosis of secondary gill lamellae, massive loss of filament tissues, compression of epithelium and thinning of tissue with loss of normal gill structure when examined with scanning electron microscopy. These changes were occasionally accompanied by localised necrosis of the epithelium.

Similar of these changes in gill epithelia of *Dicentrarchus labrax* were ultrastructurally observed by (**Giari *et al.*, 2007). Nathan *et al*., (2013)** observed ultrastructural damage such as hypertrophy, hyperplasia, erosion, desquamation and necrosis of secondary lamellae in the gills of *Mugil cephalus.* Copepods attach by using various appendages modified for grasping, and this ectoparasite activity can lead to secondary infection by pathogenic organisms (e.g. bacteria, fungi) and also to the development of adhesions between gill filaments (**Nigrelli 1950, Reichenbach- Klinke and Elkan 1965**).

Consequently, fish respiration is impaired and reduced feeding, weight loss, and general deterioration of health can result (**Reichenbach- Klinke and Elkan 1965**). There are few quantitative reports concerning the extent of pathology caused by crustaceans in wild fish populations (**Roubal 1999**).

Compared with the previous results, the present study observed damage such as cell proliferation, erosion, and necrosis of secondary lamellae in the gills of *Dicentrarchus labrax* and *Mugil cephalus* at the transmission electron microscope level. Furthermore, Observations at TEM level in the present study showed that disruption, degeneration and mucous secretion cover epithelial surface of the gill filaments of *Dicentrarchus labrax* and *Mugil cephalus.*

English Summary and Conclusion

English Summary and Conclusion

As in many parts of the world, aquaculture production in the Mediterranean Sea has been expanding rapidly over recent years. Due to the economic importance of the marine fishes in Egypt, it was necessary to investigate the important parasitic copepods that infesting these economic host fishes; *Dicentrarchus labrax* and *Mugil cephalus.*

The present study has revealed that two parasitic copepods *Lernanthropus kroyeri* belonging to genus *Lernanthropus* and *Ergasilus versicolor* belonging to genus *Ergasilus* infest the gills of the economic important marine fishes, *Dicentrarchus labrax* and *Mugil cephalus*, respectively.

- **From this point, the present thesis was planned to study host-parasite relationship from different biological aspects:-**

1- Morphological and anatomical studies of the parasitic copepods; *Lernanthropus kroyeri* and *Ergasilus versicolor*.

2- Structure of tegument and cuticular outgrowths on the body surface of the parasitic copepods; *Lernanthropus kroyeri* and *Ergasilus versicolor*.

3- Histology of the normal (non-infested) and histopathological changes of infested gills by using both scanning and transmission electron microscopy.

4- Aspects of the pathology and infection caused by *Lernanthropus kroyeri* by using both scanning and transmission electron microscopy.

5- Aspects of the pathology and infection caused by *Ergasilus versicolor* by using both scanning and transmission electron microscopy.

MORPHOLOGICAL AND ANATOMICAL STUDIES

A detailed on morphological and anatomical study has been made on the gill parasite copepods, *Lernanthropus kroyeri* and *Ergasilus versicolor* using both light and electron microscopy.

Studies with both light and electron microscopes revealed that:-

1) Light microscopy studies:

1) *Lernanthropus kroyeri*

- **Female**

1. Body of female *L.kroyeri* is elongated with long egg sacs. The cephalon and first thoracic segment fused to form cephalothorax, slightly wider than long. The remaining thoracic segments fused forming genital complex. Inside the genital complex there are two oval dorsal ovaries and large ventral cement glands.
2. Dorsal shield of genital complex in female expands posteriorly and dorsally, forming a sac with a supporting dorsal layer completely covering abdomen. Abdomen is short and distinguished at beginning of dorsal plate extension.
3. First antenna is seven-segmented while the second antenna is two-segmented ended with strong claw for attachment of parasite onto the host tissue.
4. Third thoracic leg is unarmed and long, protruding posteroventrally from medial region of genital complex, parallel to each other. Fourth thoracic leg is bilobed and unarmed, protruding ventrolaterally from distal region of genital complex.

- **Male**

1. Body of male parasite is elongated. Cephalon and first thoracic segment fused to form cephalothorax, slightly wider than long. The head separated by a constriction from the rest of the body. The remaining thoracic segments fused forming genital complex. Genital complex is slightly identical in length and width. Uropod is fusiform and unarmed. Genital complex indistinguishably fused to trunk anteriorly and to abdomen posteriorly.
2. Abdomen is short and could not be clearly delimited in male. There are two spermatophores in posterior vasa deferentia inside the abdomen of males.
3. First antenna is seven-segmented. Second antenna is sturdy and two-segmented. Maxilliped is subchelate, corpus stout unarmed and claw apicaly directed with longitudinal ridges. Uropod is unsegmented and fusiform. Fourth thoracic leg is bilobed and unarmed, protruding ventrolaterally from distal region of genital complex.

2) *Ergasilus versicolor*

1. The female body consists of two main parts, prosome and urosome. The prosome consists of cephalosome and mesosome. The first somite of the mesosome is fully incorporated into the cephalosome forming cephalothorax which is equal approximately in length to the remaining part of the body.
2. The cephalothorax is oblong, uninflated and bullet-shaped. Its anterior end is slightly tapering with slightly projecting antennary region forming a short rostrum and the posterior margin is transversely truncated. The

cephalothorax decreases in width posteriorly.

3. The mesosome consists of three free somites comprising second, third and fourth thoracic somites. There are a pair of elongated, maggot-shaped, multiseriated egg sacs are originated one from each ventro-lateral side of the genital somite.

2) *Scanning electron microscopy studies:*

1) *Lernanthropus kroyeri*

- **Female**

1. Body surface ventrally ornamented with patches of setules and elongated with long egg sacs. Cephalothorax with dorsal shield slightly is narrower anteriorly, anterolateral corners are more rounded than posterolateral corners in dorsal view.

2. The abdomen is short and one or two segmented. Inside the abdomen of females, two spermatophores sacs attach at each vaginal opening. Egg strings is usually long trailing behind the body from genital segment and uniseriate with numerous disc-shaped eggs. Each string emerged from a genital orifice containing 72 (66-80) disc-shaped eggs. Caudal rami(=uropods) is unsegmented, terminal and fusiform with 5 setules (two terminal and three subterminal).

- **Male**

1. The Body of male *L. kroyeri* is smaller than female. Head (cephalothorax) is oblong and wider than long with antennal region set apart from rest of head. Genital complex indistinguishably fused to trunk. Abdomen of male is restricted and stumpy. Caudal ramus (uropods) is long and slender.

2. First antenna seven-segmented; first segment with one seta, second segment with three setae, third with short seta, fourth with one short and two long setae, fifth with one seta, sixth with two setae, seventh with eight terminal setae. Parabasal flagellum with broader base and is pointed distal part.

3.In both female and male *L. kroyeri*, the first maxillae were bilobate and ended by two horny spines and a setule cover. The second maxillae were uniramous with a distal calamus claw armed with two sharp denticles. Maxillipeds appeared with a robust and terminal claw. The first and second thoracic legs were smaller than the other thoracic appendages and ended with hand fingers-like spines. This structure is thought to serve in the attachment to the adjacent secondary gill lamellae and to increase the parasite stability. The third and fourth thoracic legs were the largest appendages and appeared free of any cuticular structures. This unique structure is suggested to serve in adjusting the parasite position and in providing tight attachment.

4. The second antenna of *Lernanthropus kroyeri* is characteristically prehensile and uncinate and thus provides the main force for the attachment to the host tissue. The assisting action in the process of attachment is thought to be achieved by first maxillae, second maxillae, maxillipeds and the first four thoracic legs.

2) *Ergasilus versicolor*

1.The body of female parasite is slender, elongated and gradually narrowed posteriorly. It is commonly known as the ‘gill maggot’ due to the presence of long white egg sacs that trail behind the body. The parasite is usually attached towards the ends of the gill arches by the robust 2nd antennae.

2. The body form of female parasite is long and narrow. The female body consists of four regions; cephalothorax, free thorax, genital segment and abdomen.

3. Cephalothorax has one pair of antennules and one pair of antennae. The antennules are segmented and stumpy. The antennae are long, segmented and firm prehensile. Each one consists of four segments. These antennae have powerful muscles and roughened surfaces. Only the 2nd antenna plays a role in the primary attachment to gill filaments. The assistant action of attachment is made by the four swimming legs.

4. Free thorax contains five pairs of thoracic swimming legs. The first pair of thoracic legs consists of a coxapod and basipod that ornamented on their anterior surface with rows of spinules. The second and third thoracic legs are closely similar. The fourth thoracic leg consists of a coxapod that ornamented on its anterior surface with rows of spinules arranged in scattered groups and a basipod which ornamented also on its anterior surface with a single row of spinules. The extremely reduced fifth thoracic swimming leg is represented by papillary process.

5. The abdomen consists of three somites. These somites are wider than long, with almost similar width and slightly diminish posteriorly. The first abdominal somite is larger than the following two. The second abdominal somite is notched posteriorly almost up to the half of its length. The last somite is almost equal to or slightly smaller than the second one and carries a single caudal ramus. Each caudal ramus is armed distally with four terminal setae.

3) *Transmission electron microscopy studies:*

1) *Lernanthropus kroyeri*

1.The body tegument of *Lernanthropus kroyeri* shows two types of epicuticular formations:

i-Very abundant ones with a tubular or filamentous aspect.

ii-The other type of cuticular outgrowths consists of longer and more ramified expansions less abundant than the other ones.

2.The cuticle is thick and shows three different zones : epicuticle, exocuticle, and endocuticle . It is crossed by a great number of canalicles, which allow the passage of substances from the underlying epithelium. The epidermis comprised a single layer of cells separated from the endocuticle by an electron-dense apical membrane which was often elaborated into rugose folds.

3. Numerous sensory endings identified on body surface of *L. kroyeri* are involved with feeding and attachment. Also, provided the copepod with a considerable increase of cuticular surface in order to a better oxygen utilization, and so improving respiratory processes through the integument.

4. Cuticular differentiations play a secondary role in the fixation and they have mainly a sensory function. The cuticle of *Lernanthropus kroyeri* provides the principal interface between the organism and its external environment.

2) *Ergasilus versicolor*

1. The cuticle consisted of three recognisable zones. These comprised a multi-layered external epicuticle, exocuticle and an internal endocuticle from outermost to innermost. The cuticle of the dorsal body surface was generally thicker than that of the ventral body surface.

2. The body wall is equipped with a very strong chitinous layer and a thick

epidermis. The cuticle in all of these body areas is flexible. There was no indication of any cuticular outgrowths or papillae of cuticular surface.

3. There is no outgrowth or sensory structures on the body surfaces of *E. versicolor*. This indicates that the entire body surface is involved in exchange of gases, confirming earlier assumptions.

HISTOPATHOLOGICAL STUDIES

The present study represents that there were histopathological impacts of the parasitic copepods; *Lernanthropus kroyeri* and *Ergasilus versicolor* that infesting *Dicentrarchus labrax* and *Mugil cephalus* respectively, at the level of the light microscope, scanning and transmission electron microscopes.

1) *Lernanthropus kroyeri*

- **At light microscope level,** *Lernanthropus kroyeri* were primarily found attached to the ventral surface of the gill filaments, tight to the interbranchial septum . Attachment involved use of the parasite's antennae to grasp the gill filaments. Attachment was characterised by the antennae directed in a forward position toward the gill arch. Histologically, at the site of parasite attachment, regressive phenomena prevailed: complete superficial tissue erosion with exposure of the primary lamellae cartilage, exposure of blood vessels and hyperplasia resulting from the grasping action of the 2nd antennae and maxillipeds. Primary lamellae erosions, necrosis, epithelial erosion, compression and haemorrhage resulted from parasitic infection. During most infections, pathological changes were localised, leaving the majority of the gill filament relatively normal. There were also lamellary edema, fusion of the secondary lamellae due to significant epithelial proliferation, mucus cell proliferation, erosion of the branchial lamellar epithelium and necrosis in tips of the primary lamellae where the parasites penetrate.

- **Scanning electron microscopy (SEM)** observations provided useful information on the mode of *Lernanthropus kroyeri* attachment. Specifically, parasites anchored themselves to the primary lamellae through the piercing action of their second antennae. At the site of parasite attachment, regressive phenomena prevailed: superficial tissue erosion with exposure of the primary lamellae cartilage, resulting from the grasping action of the 2nd antennae, 2nd maxillae and the maxillipeds. A lesion associated with female *Lernanthropus kroyeri* was compatible with depression, destruction and massive loss of filament tissues.
- **At transmission electron microscope level,** lesions were observed with female *Lernanthropus kroyeri* with *Dicentrarchus labrax* gill filaments. Typically these growths were most pronounced about the anterior of the copepod's cephalothorax and especially just anterior to the frontal plates. The papillomatous growths associated with the attachment of female *Lernanthropus kroyeri* were characterized by marked epithelial hyperplasia with disorganization of epithelial layers, necrosis and massive loss of filament tissues. Erosion, desquamation, necrosis and degeneration of infected gill tissues and number of mucous cells were occurred at the TEM level.

2) *Ergasilus versicolor*

- **At the light microscope level,** female *Ergasilus versicolor* was found to induce histopathological changes at the site of attachment of the gill lamellae of *Mugil cephalus*. The attachment usually involved the 2nd antennae grasping two gill filaments. In most infections, the anterior regions of *Ergasilus versicolor* maintained close contact with host tissues, whilst the posterior regions of the body, including swimming thoracic legs and urosome caused

little damage. At the light microscope level, the crustacean parasite (*Ergasilus versicolor*) exerts a compression against the gill tissue at the site of attachment to the gill lamellae of *Mugil cephalus***,** with the exception of mild distortion, epithelial compression and fusion of primary filaments.

- **At scanning electron microscope level,** the major features of female *Ergasilus versicolor* infections on the gill filaments of the fish studied include destruction and erosion of the gill filaments. Mucus secretion were found cover epithelial surface of the primary gill lamellae. Attachment involved use of the parasite's antennae to grasp the gill filaments. This brought the mouth-parts located on the underside of the body into close contact with the gill tissue. Many of the pathological changes associated with *Ergasilus versicolor* resulted from attachment pronounced compression of epithelium and thinning of tissue with loss of normal gill structure.

- **At transmission electron microscope level,** the parasitized gills, enhanced mucous production, congestion, haemorrhages and secondary lamellae erosions were encountered. Erosion, desquamation and necrosis of secondary lamellae. Histopathological changes of the gill lamellae of *Mugil cephalus* included desquamation, degeneration, mucous production and hyperplasia of infected gill tissues.

In conclusion, The present study provides a contribution to our understanding of the parasitic copepods infesting important economic fishes in Egypt. Since crustacean parasites are potential pathogens in fish farms, the knowledge gained by these studies may be valuable in any attempt to raise these fishes on a commercial basis for food.

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Arabic Summary

الملخص العربى

عنوان الرسالة: دراسات على مجدافية الأرجل المتطفلة على الأسماك الاقتصادية الهامة باستخدام تقنيات حديثة.

رسالة دكتوراه مقدمة من

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نظرا للأهمية الإقتصادية للأسماك البحرية والتي تمثل مصدرا هاما من مصادر البروتين الحيواني في مصر وبالإضافة إلى التأثير الضار التي تسببه الطفيليات مجدافية الأرجل على صحة هذه الأسماك الأمر الذي يؤدي بالإضرار بالثروة السمكية فقد استهدفت الرسالة الحالية القيام بعمل دراسات على الطفيليات مجدافية الأرجل التي تصيب نوعين من الأسماك البحرية ذات الأهمية الإقتصادية وهي أسماك القاروص (دايسنتراركس لابركس) و البوري (ميوجل سيفالس) والتي تم تجميعها من المياه الساحلية لمدينة دمياط (ميناء دمياط) على البحر المتوسط. حيث أظهرت الدراسة الحالية أن هناك نوعين من الطفيليات مجدافية الأرجل (ليرناثروبث كرويري) و (ارجسيلس فرسيكلر) تم عزلها من الخيوط الخيشومية لتلك الأسماك على التوالي.

اهتمت الدراسة الحالية بموضوعين أساسيين الموضوع الأول تناول النواحي المورفولوجية والتشريحية للطفيليين مجدافي الأرجل. الموضوع الثاني تناول فهم كيفية الالتصاق لهذين الطفيليين والآثار الهستوباثولوجية المرضية الناتجة عن هذا الالتصاق باستخدام الميكروسكوب الضوئي وتقنية الميكروسكوب الالكتروني الماسح والنافذ وقد أسفرت الدراسة عن النتائج التالية:

أولا: الدراسات التصنيفية والمورفولوجية والتشريحية

بينت الدراسة الحالية أن هناك نوعين من الطفيليات مجدافية الأرجل تصيب خياشيم أسماك

القاروص (دايسنتراركس لابركس) والبوري (ميوجل سيفالس) المستوطنة في المياه الساحلية لمدينة دمياط (ميناء دمياط) على البحر المتوسط وهى: ليرناثروبث كرويري على أسماك القاروص و ارجسيلس فرسيكلر على أسماك البوري و قد احتوت هذه الدراسة على عدة نقاط مهمة وهى شكل و أجزاء الجسم بالإضافة إلى زوائد الجسم وكذلك طبقات جدار الجسم لكلا الطفيليين على مستوى الميكروسكوب الضوئي العادي وباستخدام الميكروسكوب الالكتروني الماسح و مستوى الميكروسكوب الالكتروني النافذ. و أظهرت الدراسة الحالية النتائج التالية:-

أ) أجزاء الجسم

- **طفيلي (ليرناثروبث كرويري):**

1- الجسم في أنثى وذكر (ليرناثروبث كرويري) ممدود ، وتتحد منطقة الرأس مع عقل الصدر الأولى مكونة الرأسصدر ثم تتحد العقل الأخيرة من منطقة الصدر لتكون المجمع التناسلي.

2- يمتد في نهاية جسم الأنثى أكياس البيض الطويلة ، و بداخل مجمع الأعضاء التناسلية في أنثى طفيلي (ليرناثروبث كرويري) يوجد زوج من المبايض بيضاوية الشكل ظهرية الموضع، وأيضا زوج من الغدد الأسمنتية كبيرة الحجم بطنية الموضع.

3- البطن في أنثى وذكر طفيلي (ليرناثروبث كرويري) قصيرة و مميزة وواضحة في الأنثى عن الذكر، و يوجد زوج من أمهات المني في الأوعية الناقلة الخلفية داخل البطن في الذكر.

4- عادة ما تكون أكياس البيض في أنثى (ليرناثروبث كرويري) طويلة و زائدة خلف الجسم وتمتد من العقدة التناسلية وبداخلها العديد من البيض القرصي الشكل. ينشأ كل كيس من الدهليز التناسلي محتويا على البيض الذي يتراوح عدده من 66-80 بيضة.

5- تظهر الذؤابات الذيلية (الأرجل الذيلية) في أنثى (ليرناثروبث كرويري) غير معقلة وطرفية ومغزلية الشكل وعليها 5 شويكات (اثنين طرفية وثلاثة تحت طرفية) أما في الذكر تكون الذؤابات الذيلية طويلة ونحيلة.

- **طفيلي (ارجسيلس فرسيكلر):**

1-يتكون الجسم في طفيلي (ارجسيلس فرسيكلر) من جزأين رئيسيين مقدمة الجسم ونهاية الجسم. يتكون مقدمة الجسم بدوره من مقدمة الرأس و وسط الجسم. تندمج العقلة الأولى من وسط الجسم مع مقدمة الرأس مكونة الرأسصدر والتي يتساوى طولها تقريبا الجزء المتبقي من الجسم.

2- تظهر منطقة الرأسصدر مستطيلة وتكون النهاية الأمامية لها مستدقة مكونة بروز قصير ويقل الرأسصدر في العرض خلفيا.

3- يوجد زوج من أكياس البيض الممدودة دودية الشكل وينشأ كل كيس من الدهليز التناسلي. تحتوي أكياس البيض على العديد من البيض كبير الحجم وكروي الشكل ويتراوح عدده من 87-90 بيضة يمكن رؤيته بوضوح من خلال الغشاء الرقيق لكيس البيض.

ب) *زوائد الجسم*

- **طفيلي (ليرناثروبث كرويري):**

تمت دراسة زوائد الجسم في الطفيلي مجدافي الأرجل (ليرناثروبث كرويري) على مستوى الميكروسكوبين الضوئي العادي و الالكتروني الماسح. وقد أوضحت الدراسة أن:-

1- الزباني الأول في أنثى وذكر طفيلي (ليرناثروبث كرويري) يتكون من سبع عقل بينما الزباني الثاني في كلا الجنسين عبارة عن عقلتين وينتهي بمخلب قوي وله دور أساسي وأصيل في إلتصاق الطفيلي بأنسجة العائل. بالإضافة إلى وجود زوج من زوائد السوط المجاورة والتي تكون قاعدتها عريضة وواسعة وتنتهي بنهاية مدببة.

2- الزوج من الفك الأول "الفكيك" ذو فصين؛ و يكون الفص الداخلى أصغر من الخارجي و ينتهي هذا الفك بأشواك قرنية طرفية وغطاء شويكي.

3- وجود زوج من الفك الثاني " الفك" يلي الأول وهو وحيد الشعبة وينتهي بمخلب قوي و

صفين من الشويكات الصغيرة الحادة. وتم اقتراح أن الفك الأول والفك الثاني يقومان بدور ثانوي في عملية إلتصاق الطفيلي بأنسجة العائل.

4- وجود زوج من الأرجل الفكية القوية والتي تنتهي بمخلب طرفي ولها حافة طويلة مسننة تلي زوج الفك الثاني وتساعد أيضا في عملية إلتصاق الطفيلي بأنسجة عائله.

5- الزوائد السابقة تليها أربع أزواج من زوائد الأرجل الصدرية وقد بينت الدراسة أن الأرجل الصدرية الأولى والثاني صغيرة ويختلف تركيبها عن الزوائد الصدرية الأخرى حيث أنها تنتهي بأشواك تشبه أصابع اليد. لذا اقترحت الدراسة أن لتلك الزوائد الاصبعية لها دور مساعد يفيد الطفيلي في الارتكاز على الصفائح الخيشومية الثانوية المجاورة مما يتيح زيادة ثبات واستقرار الطفيلي على أنسجة العائل.

6- الأرجل الصدرية الثالثة والرابعة وهي أكبر الزوائد؛ لها شكل ورقي بدون أي هياكل أو تراكيب جليدية أو صلبة. واقترحت الدراسة أن هذا التركيب الفريد يساعد في ضبط مكان وموضع الطفيلي بالإضافة إلى دورها في الإلتصاق المحكم للطفيلي على الخيوط الخيشومية للعائل. كما لوحظ أن تركيب وشكل تلك الأرجل الطويلة يتلاءم مع بيئة الطفيلي "الخيوط الخيشومية" مما قد يفيد في مقاومة الطفيلي لتيار الماء التنفسي الداخل إلى بيئته وتثبته على أنسجة العائل.

7- الزباني الثاني بما له من تركيب فريد يمكن أن يقوم بالدور الأساسي في عملية الإلتصاق بينما يمكن أن تقوم باقي الزوائد (الفك الأول والثاني والرجل الفكية والأرجل الصدرية الأربعة) بالدور الثانوي في عملية إلتصاق الطفيلي بأنسجة العائل.

- **طفيلي (ارجسيلس فرسيكلر):**

تمت دراسة زوائد الجسم في الطفيلي مجدافي الأرجل (ارجسيلس فرسيكلر) على مستوى الميكروسكوبين الضوئي العادي و الالكتروني الماسح. وقد أوضحت الدراسة أن:-

1-الجسم في طفيلي (ارجسيلس فرسيكلر) رفيع، ممدود ويضيق تدريجيا للخلف وهذا يتلاءم مع تسمية جنس ارجسيلس بـ "الدودة الخيشومية" بسبب وجود أكياس البيض البيضاء الطويلة التي تمتد وراء الجسم.

2-شكل الجسم في طفيلي (ارجسيلس فرسيكلر) طويل وضيق؛ ويتكون من أربع مناطق: الرأسصدر، الصدر الحرة، المجمع التناسلي والتي تمثل "مقدمة الجسم" والبطن والتي تمثل "نهاية الجسم".

3-منطقة الرأسصدر تحتوي على زوج واحد من الزباني الأول "الزبيني" وزوج واحد من الزباني الثاني "الزباني". الزباني الأول معقل وقصير بينما الزباني الثاني طويل ومعقل و يظهر كأعضاء ماسكة قوية.

4-كل زباني مكون من أربع عقل مدعمة بعضلات قوية وأسطح مسننة وينتهي كل زباني بمخلب قوي.اقترحت الدراسة أن الزباني الثاني فقط يمكن أن يقوم بالدور الأساسي في عملية الإلتصاق بالخيوط الخيشومية لطفيلي (ارجسيلس فرسيكلر) بينما تقوم الأرجل الصدرية السباحة الأربعة بالدور الثانوي في عملية الإلتصاق.

5-منطقة الصدر الحرة في طفيلي (ارجسيلس فرسيكلر) تحتوي على 5 أزواج من أرجل العوم الصدرية. يتكون الزوج الأول من أرجل العوم الصدرية من شدفتين: الشدفة الحرقفية والشدفة القاعدية وتتزين الشدفات على سطحها الأمامي بعدد من الشويكات الصغيرة. الزوج الثاني والثالث متشابه بشكل وثيق بينما الزوج الرابع يحتوي على صفوف من الشويكات الصغيرة مرتبة في مجموعات متفرقة على السطح الأمامي للشدفة الحرقفية و صف واحد من الشويكات الصغيرة للشدفة القاعدية. أما الزوج الخامس من أرجل العوم الصدرية فهو مختزل جدا ويتمثل في زائدة حليمية.

6-البطن مكون من ثلاثة عقل عرض كل عقلة أطول من طولها. فالعقلة البطنية الأولى كبيرة عن العقل التالية بينما العقلة الثانية تمتد للخلف وعرضها يساوي طولها تقريبا. أما العقلة الأخيرة يتساوى عرضها مع طولها ولكنها أصغر من الأولى والثانية وتحمل الذؤابات الذيلية وحيدة الشعبة وتنتهي كل ذؤابة بأربع أشواك طرفية.

ت) طبقات جدار الجسم

تمت دراسة طبقات جدار الجسم في الطفيلي مجدافي الأرجل (ليرناثروبث كرويري) باستخدام الميكروسكوبين الضوئي العادي و الالكتروني الماسح والنافذ. وأظهرت الدراسة أن:-

- **طفيلي (ليرناثروبث كرويري):**

1- جدار الجسم من الخارج في طفيلي (ليرناثروبث كرويري) يحتوي على تركيبات أو بروزات جليدية.

2- جدار الجسم في طفيلي (ليرناثروبث كرويري) يتكون من طبقتين: الجليد والبشرة. يحتوي الجليد على ثلاث مناطق مختلفة ترتيبهم من الخارج إلى الداخل كالأتي: الجليدة الفوقانية، الجليدة الخارجية والجليدة الداخلية و التي يعبر من خلالها عدد من القنوات الصغيرة "القنيات" التي تسمح بمرور المواد إلى طبقة البشرة. تتألف طبقة البشرة من طبقة واحدة من الخلايا يفصل بينها وبين الجليدة الداخلية غشاء قمي كثيف.

3- العديد من النهايات الحسية التي تم تحديدها على سطح الجسم في طفيلي (ليرناثروبث كرويري) يمكن أن تشارك في التغذية و عملية الإلتصاق و علاوة على ذلك تقوم النهايات الحسية بتحسين الاستفادة من الاكسجين لعملية التنفس خلال الإهاب السميك للطفيلي (ليرناثروبث كرويري).

- **طفيل (ارجسيلس فرسيكلر):**

1-أثبتت الدراسة أن جدار الجسم من الخارج في طفيلي (ارجسيلس فرسيكلر) لا يحتوي على تركيبات أو إمتدادات أو بروزات جليدية مما يتضح أن سطح الجسم بأكمله يشارك في تبادل الغازات.

2- جدار الجسم في طفيلي (ارجسيلس فرسيكلر) يتكون من طبقتين: الجليد متعدد الطبقات

والبشرة. يحتوي الجليد على ثلاث مناطق مختلفة؛ الجليدة الفوقانية، الجليدة الخارجية والجليدة الداخلية على الترتيب من الخارج للداخل.

3- الجليد الذي يغطي السطح الظهري للجسم أكثر سمكا من السطح البطني. ويلي طبقة "الجليد الكيتينية السميكة" طبقة البشرة السميكة والتي تحتوي على نوع واحد من الخلايا الطلائية ويفصلها عن الجليد غشاء كثيف.

ثانياً: الدراسات النسيجية والآثار الهستوباثولوجية

تم دراسة التركيب النسيجي الطبيعي (الغير مصاب) لخياشيم العوائل السمكية القاروص (دايسنتراركس لابركس) والبوري (ميوجل سيفالس) موضع الدراسة باستخدام الميكروسكوب الضوئي العادي والميكروسكوب الاليكتروني الماسح والنافذ. و قد أظهرت الدراسة وجود أنواع كثيرة من الخلايا منها الخلايا الكلوريدية، الخلايا المخاطية بالإضافة إلي الخلايا الطلائية التي تغطي الخيوط الخيشومية الأولية و الخلايا الدعامية التي تدعم الصفائح الخيشومية الثانوية.

أظهرت الدراسة الحالية أن التأثيرات المرضية الناجمة عن الإصابة بالطفيليات مجدافية الأرجل (ليرناثروبث كرويري) و (ارجسيلس فرسيكلر) باستخدام الميكرسكوب الضوئي و الاليكتروني الماسح والنافذ تمثلت في تآكل الأنسجة السطحية للخيوط الخيشومية الأولية، ونمو غير طبيعي لخلايا النسيج الخيشومي، تضخم الخيوط الخيشومية الاولية، إلتصاق في الصفائح الخيشومية الثانوية المجاورة لمكان الإلتصاق.

بالإضافة لذلك تغير في شكل الخيط الخيشومي، وزيادة الإفراز المخاطي وتآكل وتمزق الأنسجة الخيشومية وإتلافها خاصة حول مكان الالتصاق. لوحظ عدد من الإصابات المرضية الأخرى مثل: تقشر في الخيط الخيشومي، نخر وتآكل النسيج الخيشومي و وجود عدد من الخلايا المخاطية.

أثبتت الدراسة عملية الإلتصاق لطفيلي (ليرناثروبث كرويري) على مستوى الميكروسكوب الالكتروني الماسح وبينت أن طفيلي (ليرناثروبث كرويري) يرتكز على الصفائح الخيشومية الأولية بواسطة زوج الزباني الثاني بمخلبه القوي ويظهر عند مكان الإلتصاق تآكل وضغط على غضاريف الصفائح الاولية بالإضافة إلى الزوائد الأخرى "الفك الاول والثاني والرجل الفكية" المسببة لخسائر وتقشر في الصفائح الخيشومية.

أوضحت الدراسة الحالية أيضا عملية الالتصاق لطفيلي (ارجسيلس فرسيكلر) على الخيوط الخيشومية للعائل. حيث كان الزباني الثاني هو أيضا المسئول عن إلتصاق الطفيلي للصفائح الأولية بالإضافة إلى الدور المساعد في الإلتصاق عن طريق الأربع أزواج من أرجل العوم. أدى ذلك إلى العديد من التغيرات المرضية مثل الضغط على طلائية الصفائح الخيشومية وترقق الأنسجة مع فقدان هيكل أو بنية الخيشوم الطبيعي.

وفي الختام تقدم هذه الدراسة مساهمة في فهم للمجدافيات الطفيلية التي تصيب الأسماك الاقتصادية المهمة في مصر. حيث أن الطفيليات القشريات هي مسببات الأمراض المحتملة في المزارع السمكية، والمعرفة المكتسبة من هذه الدراسات قد تكون ذات قيمة من أجل الحفاظ على هذه الأسماك حيث أنها مصدر غذاء هام للإنسان.

جامعة دميـــــأط
كلية العـــــــلوم
قسم علـم الحيــوان

دراسات على مجدافية الأرجل المتطفلة على الأسماك الاقتصادية الهامة باستخدام تقنيات حديثة

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