University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 14, Issue 1 (2025) PP 162-172

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/utjagr.v13i1.323*

Morphological and molecular study of *L.cyprinacea In Cyprinus carpio* from Aghjalar region in Sulaimaniyah province

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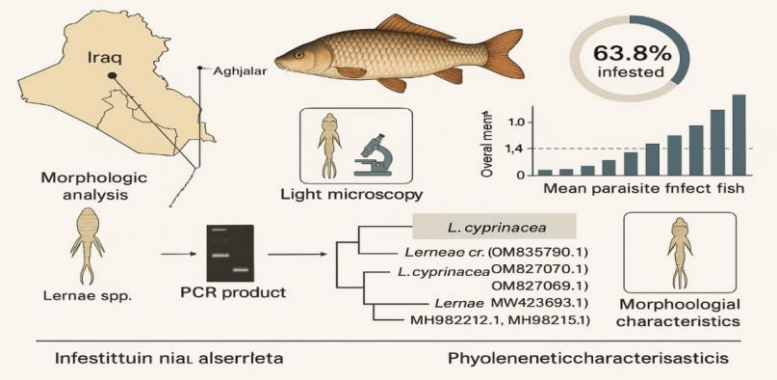
Abstract

The aim of the study Identifying key *Lernaea cyprinacea* affecting aquaculture systems and their host-specific interactions in Agjalar region in Sulaimaniyah province Kurdistan region-Iraq. In this study total of 80 fish (*Cyprinus carpio*) have been collected. Morphological and light microscopy, as well as the sequencing analysis of (28S rDNA), were used to describe *Lernaeacyprinacea*in this study. 51 individuals (63.8%) were found to be infested with the ectoparasite Lernaea spp. The overall mean parasite load per infected fish was extremely high, recorded at 14.7 parasites per host, but individuals with even higher numbers of parasites also exist. Molecular analysis results indicate that the PCR product of *Lernaea cyprinacea* was 715 bp recorded the highest similarity with (OM835790.1, OM827070.1, OM827069.1, MW423693.1, and MW423694.1) with homology 100%.Our phylogenetic analyses showed that the taxonomic position of the species *L. cyprinacea*is closely related to *Lernaea cyprinacea*(OM835790.1, OM827070.1, OM827069.1, MW423693.1 and MW423694.1) and *Lernaea cruciata*(MH982212.1 and MH982215.1).

Keywords: Molecular phylogenetics, Lernaeac cyprinacea, Cyprinus carpio, Parasite prevalence, 28S rDNA sequencing



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I. Introduction

Fish consists of a large number of species of widely differing appearances (Othman et al., 2023; AbdulRahman et al. 2012). Fish are freshwater and saltwater finfish, crustaceans, mollusks, and other aquatic animals intended for human consumption (Othman et al., 2024; Albashr et al., 2022), is a vital and vital food for human survival (Albashr et al., 2024; Hamaamin & Khidhir, 2022). Fish from some areas of the world can carry microorganisms that are harmful to human health (Khidhir, 2022; Khashroum, 2024).

Outbreaks of contagious diseases represent an essential issue in aquaculture, causing serious economic losses when not properly controlled (Piasecki et al., 2004). Infectious disease outbreaks usually arise when there is an imbalance among the aquatic environment, host, and pathogenic microorganisms. Stress in fish can be caused by poor aquaculture practice, leading to weakened defenses and an increase in infection risk (Noga , 2010; de Freitas Souza et al., 2019).Parasite infections are generally common in freshwater fish. They can be inhibitive to growth, can reduce reproductive viability, and induce mortality (Bilal et al., 2024). One parasitic disease of considerable impact is lernaeosis due to copepod ectoparasites from the genus Lernaea (Hua et al., 2019). They adhere externally to different areas of the fish and, in extreme cases, penetrate internal structures such as the mouth and gills (Noga , 1986; Barson et al., 2008), such as gill filaments and eyes (Woo et al., 2006; Nur et al., 2022). Mortality in farmed fish stocks is related to lernaeosis, notably in those cases due to Lernaea cyprinacea, an introduced, widespread species (Nur et al., 2018; Zhu et al., 2021).Traditionally, identification of *L.cyprinacea* is based on the morphology of the mature females' holdfast (Prastowo et al.,2023. Yet, holdfast morphology can drastically alter following acquisition of lernaeosis, and errors arise (Hua et al., 2019). In an attempt to increase diagnostic accuracy, molecular diagnostic methods involving DNA sequencing of 18S rRNA, 28S rRNA, and cytochrome c oxidase subunit 1 (COI) genes have been introduced (Zhu et al., 2021; Song et al., 2008). The parasitic crustaceans belonging to the family Lernaeidae infest fish in both marine and freshwater environments across the globe. About 110 species have been grouped into 14 genera (Ho, 1998). The genus Lernaea belongs to the phylum Arthropoda, class Crustacea, order Copepoda, and family Lernaeidae (Hoffman, 1967). The most widespread species is Lernaea cyprinacea, which has been found in ornamental fish in North America, Europe, Asia, South Africa, and East Australia (Hoffman, 1967). Its impact on aquaculture is still increasing because of its outbreaks in ornamental fish farms across the world (Kır, 2007).

L.cyprinacea infestations affect host welfare by reducing growth rates, changing metabolic functions, and generating painful focal infestations compromising survival (Klinger, 1998). Adult females of the species *L. cyprinacea* usually attach to the host's body at the head, back, belly, and tail regions, while frequent accumulations in fins also usually occur (Adams, 1984). Improved molecular identification using 18S rRNA, 28S rRNA, and COI genes provides better species identification (Zhu et al., 2021; Song et al., 2008).

Outbreaks of lernaeosis in aquaculture ponds in Sulaimaniyah Province, Iraq, have caused reduced production and quality of fish, especially *Cyprinus carpio* (Mama, and Abdullah ,2013) . This research aimed at identifying Lernaea species infesting common carp in Sulaimaniyah Province

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using both morphological characters and molecular markers in an attempt to elucidate parasite taxonomy and distribution.

II. . Materials And Methods

2.1.study area

Northeastern Iraq's Sulaymaniyah Province is a hilly area of Kurdistan. Latitudes 35° 05' and 36° 30' and longitudes '44 25' and 46° 20' are its coordinates. It is situated on the border between Iran and Iraq. The province of Sulaymaniyah is home to a variety of aquaculture farms. Fish farms in the Goptapa affiliated with theAghjalar district served as the study's site Latitude35.84442, longitude44.83204 coordinates of the Aghjalar region (Google map)(figuer1)

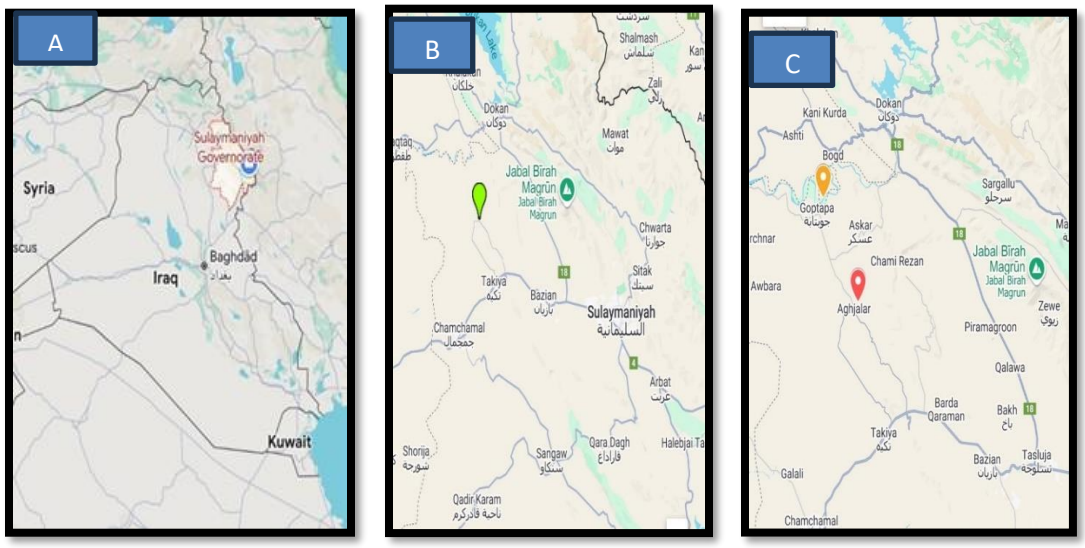

Figure-1-A- Map of Iraq, showing Sulaymaniyah Province.B- Map showing theAghjalar districtC- Map showing the study area.

2.2 Sample Collection

Sample collections and Collection of Lernaea isolates

From September to December 2024, 80 (*Cyprinus carpio*) were collected.which were captured using hand nets, gill nets, and seine nets.Fishes were put on ice and immediately transported tothe laboratory. The *Lernaea* samples were collected from commoncarp infected.The gills were isolated from the fish using blades and placed in dishes; water was poured over them, and the gill filaments were separated using a fine needle. We examined them under a dissecting microscope, where the parasites were separated and removed from the gill filaments, and we isolated them using a pipette.Smears of ectoparasites found on the skin and gills weremade by scraping the slide

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and examined under a microscope. They are fixed and cleaned in 70%ethanol (Soylu, 2005). Isolates were washed separately with 0.75% NaCl andpreserved in 95% ethanol until isolation of genomic DNA and in 4%formalin for morphological identification.The anchor shapes were studied under the microscope andconfirmed that they weremorphologically distinct fromone another. The anchor shapes were studied under the microscope, andit was confirmed that the parasite parts were observed.

2.3 Morphological identification

Anchorworm was removed from the fish using tweezers, rinsed with a saline solution, preserved in lactophenol, and mounted in Canada balsam for morphological analysis (Fernando, 1972). For molecular identification, Lernaea samples were stored in absolute ethanol. Observations focused on features such as the holdfast, body position, abdomen, and number of legs (Figure 2). Measurements of body and anchor length were also taken. Species identification of L. cyprinacea was carried out using Kabata's key from 1985.

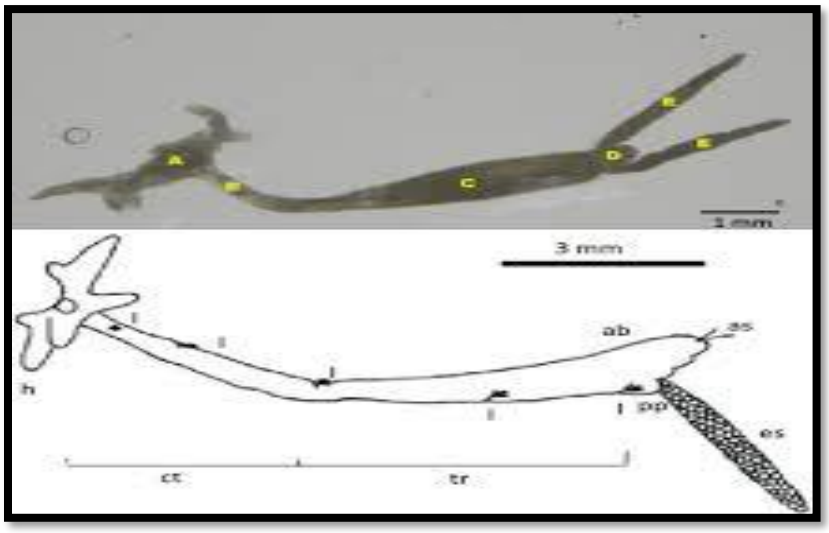

Figure-2: Comparison of holdfast morphology in Lernaeacyprinacea found in literature. *L. cyprinacea*was found in the study (left). (A) Holdfast, (B) cephalothorax, (C) trunk, (D) abdomen, and (E) egg sacs. Schematic morphology of L. cyprinacea (right): ab=Abdomen, as=Anal setae, ct=Cephalothorax, es=Egg sac, h=Head, pp=Pregenital prominence, l=Legs, tr=Trunk (Gervasoni,*et al* 2018)

2.4 Molecular analysis

2.4.1 DNA extraction

Genomic DNA extraction was used to carry out molecular identification. The parasite DNA. The gSYNC™ DNA Extraction Kit Quick procedure was employed. DNA samples had been kept at -20 °C until they were ready for use. A Nanodrop spectrophotometer was used to quantify the amount of DNA and check its quantity. *Lernaeacyprinacea Linnaeus* has a DNA amount of 1.75. The isolated DNA is identified and its quality is evaluated using 1.5% agarose gel electrophoresis.

2.4.2 Polymerase chain reaction (PCR)

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Genomic DNA was diluted to a volume of 25 µl for the PCR reaction, with 2 µl each of the primers, 12 µl of Master Mix 10 X buffer containing MgCl2, 3 l of dNTPs (10 mM), 0.9 l of 1 U of Taq DNA polymerase (Biotools, Spain), 7 µl of genomic DNA, and 5 µl of distilled water. The 28S rDNA fragments were amplified with primers 28S F(5' - ACA ACT GTG ATG CCC TTA G - 3') and 28S R (5' - TGG TCC GTG TTT CAA GAC G - 3') designed (Song et al., 2008).PCR reactions were done in a Thermal Cycler with the following PCR conditions: 94 °C for 5 min, 30 cycles of 94 °C for 30 s, 60 °C for 25 s, 72 °C for 30 s, and a 3-min final extension at 72 °C. The amplification was achieved utilizing 1.5% of agarose gel for visualization.

2.4.3 DNA sequencing and assembly

Using the same primer as above, the sequencing processes were carried out using a Genetic Analyzer 3500, Applied BioSystems (USA), according to the instructions provided by the manufacturer.The 28rDNA sequences were matched with sequences from other similarly associated species**.**The sequence was manuallychecked andedited for accuracy using BioEdit software.

2.4.4 Phylogenetic analysis

Phylogenetic trees were constructed using neighbor-joining trees method from Biotechnology Information nucleotide-BLAST (USA) (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

3. Results

Of the 80 sampled in the Aghjalar region (Chamchamal District, Iraq), 51 individuals (63.8%) were found to be infested with the ectoparasite Lernaea spp. Further, the overall mean parasite load per infected fish was extremely high, recorded at 14.7 parasites per host, but individuals with even higher numbers of parasites also exist.

Scientific classification:

Domain: Eukaryote
Kingdom: Animalia
Phylum: Arthropoda
Class: copepod
Order: Cyclopoida
Family: Lernaeidae
Genus: Lernaea
Linnaeus 1758

3.1.Morphological Lernaea identification

morphology revealed the worm, the first swimming limb is connected to a slightly round middle cephalothorax that is part of the holdfast system. The second pair was part of the neck, while the fourth pair was in the abdomen. There is no separation of parts in the body. There are two branches on the holdfast called the dorsal and the ventral. The ventral branches were simple and thin, but the dorsal branches had additional branches. An egg sac measuring between 1 and 2 mm was found in the back region of female L. cyprinacea. The examination of morphology fits the earlier description of L. cyprinacea from 1985 by kabata.

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In her youth, she is harmless: as a larva, she swims freely like a tiny shrimp, using her antennae and walking legs (Figures 3 and 4).

Figuer-3: Photomicrograph of Copepodal stage of *L. cyprinacea* (400x)

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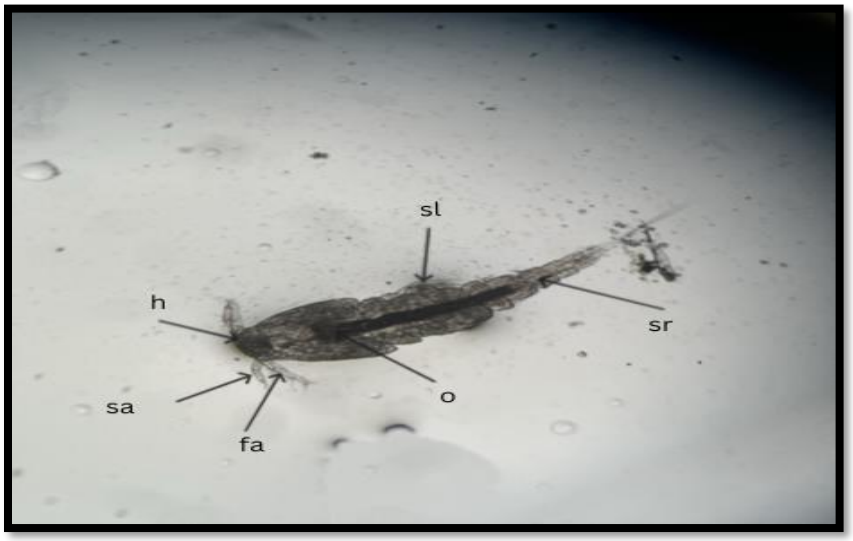

Figure-4: Photomicrograph of Copepodal stage of *L. cyprinacea* (400x)fa= first antenna; sa=second antenna; sl= swimming legs; sr=seminal receptacleo=ovary;h=head

3.2.Molecular analysis

The *28S rDNA was* amplified, the *28S rDNA* amplified a 715 bp product(Figure 5). After the manual removal of *28s rDNA*using BioEdit, the low-quality chromatogram wasdeleted. The sequences retrieved in this study were geneticallysimilar to *L. cyprinacea*, as determined by the NationalCenter for BiotechnologyInformation nucleotide-BLAST (USA) (https://blast.ncbi.nlm.nih.gov/ Blast.cgi) analysis(Ho,1998). The genetic similarity between *L. cyprinacea* and other crustaceans recorded in GenBank varied from %100 with accretion number (OM827069.1) to %88.19 with accretion number (KF153695.1).

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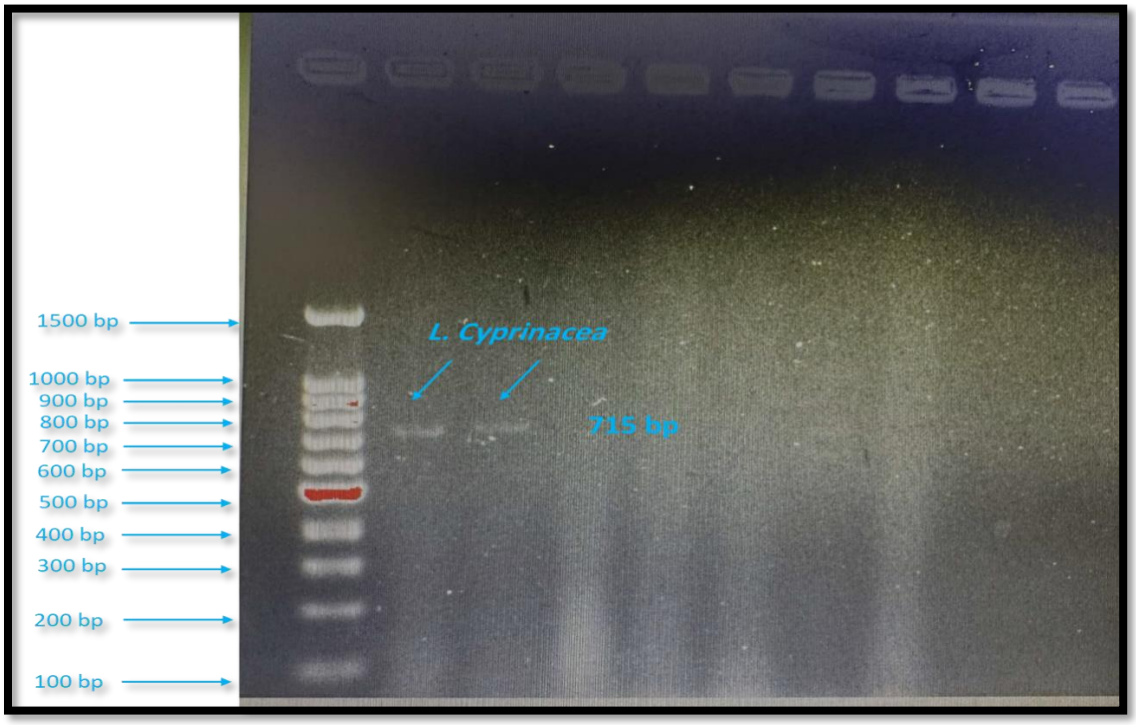

Figure -5: Picture of an agarose gel with polymerase chainreaction results from the 28S rDNA region of isolated *Lernaea parasites* on*Cyprinus carpio*. M= DNA ladder 1000 bp,gene product size 715 bp.

3.3.Phylogenetic analysis

Fig. 6 shows the phylogenetic tree relationship of *Lernaeacyprinacea*with other Lernaea species recorded in GenBank based on 18S rDNA constructed using Neighbor-Joining method from Biotechnology Information nucleotide-BLAST (USA) (https://blast.ncbi.nlm.nih.gov/Blast.cgi). High levels of genetic similarity were found in the sequence of the *28S rRNA* for *L. cyprinacea*from Sulaimani province and those from other geographic origins (Fig. 6).

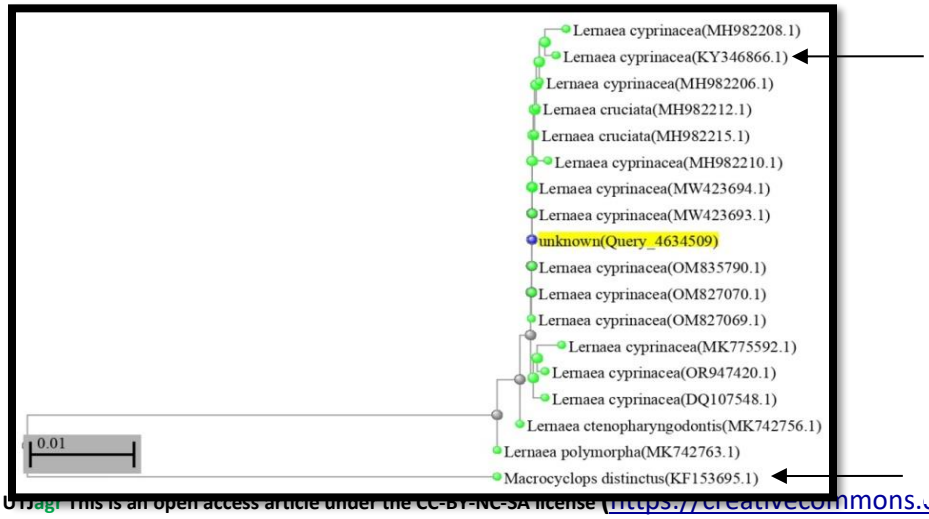

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Figure -6: Neighbor-joining phylogenetic tree constructed using the 28S rDNA. Lernaea sequences in this study were combined with*Lernaeacyprinacea*from different nations. Macrocyclopsdistinctus(KF153695.1) as our group.

III. . DISCUSSION

The parasitic copepod *Lernaeacyprinacea* exhibits distinct distribution patterns and morphological adaptations in (*Cyprinus carpio*)(Adams, 1984; Noga, 2010) across aquaculture ponds in Aghjalar district, Sulaimaniyah. Skin and gills are preferentially colonized due to their vascularized tissues, facilitating nutrient uptake and secure anchoring via the parasite's bifurcated holdfast.

Thehigh prevalence (63.8%) and intensity (14.7 parasites/host) of Lernaea spp. The Infestation observed in *Cyprinus carpio* from the Aghjalar region aligns with studies linking Lernaea (anchor worm) outbreaks to eutrophic water conditions and elevated temperatures, which accelerate parasite life cycles (Piasecki et al., 2004; Iqbal et al., 2012). The skewed distribution of parasites, with some individuals harboring extreme burdens, reflects host susceptibility variability, possibly due to stress from overcrowding or immunosuppression (Lafferty &Kuris, 1999). Such heavy infestations are known to cause epithelial damage, secondary infections, and metabolic strain, exacerbating mortality risks (Rahman et al., 2020), as noted in your study. The ecological imbalance suggested here—potentially driven by anthropogenic factors like organic pollution or unsustainable aquaculture practices—mirrors trends in other regions where water quality degradation correlates with parasite proliferation (Aly et al., 2020). However, the absence of direct water quality metrics (e.g., dissolved oxygen, and nutrient levels) in your data limits causal inferences.

Forty species of this parasite have been identified, and 32 species have been named. *L. cyprinacea* is the most important species of the Lernaea genus and has a wide host of fishes, especially common carp . Salmonidae is also infected with this parasite, although in salmon farms until 2013, this parasite had not been observed, which is most likely related to the use of water with a temperature of 14 ºC or less, especially spring water for breeding, and the only reported case was related to the population of breeding trout in floating cages in Hamon lagoon (Jalali,1998) For the first time in Iran, infection with this parasite has been reported in different parts of Iran (Jalali,1998;Abdi;Alishahi and Peighan 1999;Jalali,1987)observed this parasite in Bream, Grass carp, Crucian carp and Common carp (Jalali,1987). Significant infestations of *L.elegans* have been documented in Zarivar Lake, located in the Kurdistan Province (Jazebizadeh, 1983). In a notable case, 1,462 individual parasites were isolated from a single silver carp specimen (Alishahi & Peighan, 1999). Furthermore, widespread infections by Lernaea spp. were observed among both native and non-native cyprinid species collected from the Kor River Basin—including the Kor River and Dorudzan Reservoir—in southwestern Iran during the years 2010 and 2011 (Sayyadzadeh et al., 2011).

The findings of the present study contribute to the expanding body of literature on *L.cyprinacea*, a parasitic copepod of increasing relevance in global aquaculture. By employing both

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morphological and molecular approaches, this research underscores the limitations of conventional taxonomic methods and emphasizes the critical role of DNA sequencing in resolving ambiguities in species identification. For instance, morphological identification based on holdfast structures has historically been prone to inaccuracies due to phenotypic plasticity, which is influenced by host size, environmental conditions, and developmental stage (Hua et al., 2019; Zhu et al., 2021; Kır, 2007).

Comparative analysis of the 28S rRNA gene sequences from *L. cyprinacea* specimens obtained in Yogyakarta and other geographic locations revealed a high degree of genetic similarity, with 100% sequence identity observed across all samples regardless of their provenance. Phylogenetic reconstruction based on the 28S rDNA gene (Fig. 6) confirmed that the sequence from the present study clustered within the same clade as L. cyprinacea and *L. cruciata*. The gene sequences exhibited complete homology with previously recorded accessions (OM835790.1, OM827070.1, OM827069.1, MW423693.1, and MW423694.1) (House, C. H., and Fitz-Gibbon, S. T. ;2002).

These findings highlight the necessity for more robust molecular-based methodologies in the taxonomic delineation of *Lernaea sp*. According to Kabata (1985), approximately 37 distinct Lernaea spp. have been described; hwever, with the advancement of molecular techniques, this number is expected to decline as genetically indistinguishable taxa are re-evaluated. For example, Hua et al. (2019) proposed that L. cyprinacea and *L. cruciata* may, be conspecific. Overall, the study reinforces the value of molecular tools in parasitological research. While traditional morphological keys (Hoffman, 1967; Jalali, 1998) continue to serve as important foundational resources, molecular phylogenetic analyses—particularly those employing 28S rDNA sequences—are instrumental in resolving longstanding taxonomic uncertainties.Future research should expand genomic databases with mitochondrial markers (e.g., COI) to enhance resolution for population genetics and trace invasion pathways (Song et al., 2008). Additionally, investigating host immune responses to *Lernaea* infections could inform targeted therapies, reducing reliance on chemical treatments that risk environmental contamination (de Freitas Souza et al., 2019).

The level of infestation points to a breakout of critical ecological ineptitude, very likely to be attributed to factors such as a rise in water temperatures, overcrowding, or poor water quality. The widespread occurrence and intensity of Lernaea spp. Listed for this study are capable, when taken into consideration, of diminishing fish health leading to retarded growth, depressed immune responses, and heightened mortality. The findings, thus, provide a stark reminder of the need for developing targeted interventions to check the spread of parasites. Finally, it is pertinent to stem any further decline in the indigenous populations of fish.

IV. Conclusion

The endemic presence of *L. cyprinacea* in Sulaimani Province underscores the intersection of environmental, biological, and anthropogenic factors driving Lernaeosis in aquaculture . Addressing this challenge requires integrated strategies: optimizing pond temperatures, improving biosecurity protocols, and adopting molecular diagnostics for early detection. By contextualizing local findings within global trends, this study advocates for transnational collaboration to curb the spread of this pervasive parasite and safeguard aquatic food security. Future work should quantify

University of Thi-Qar Journal of agricultural research

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these parameters and assess seasonal variation in infestation rates to identify mitigation levers, such as reducing stocking densities or improving filtration systems.

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ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 14, Issue 1 (2025) PP 162-172

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ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 14, Issue 1 (2025) PP 162-172

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