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Morphological and Molecular Description of *Lernaea cyprinacea* (Copepoda: Cyclopoida), a Parasite of Commercially Important Cyprinus carpio Fish

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ABSTRACT

The purpose of this article is obtaining information about *Lernaea* species that infects carp fish in the fish farms of Tashkent and Fergana regions of Uzbekistan. Of the 240 carp samples examined, 76 were found to be infected with *Lernaea cyprinacea*, with the highest infection rate being 40% in the Tashkent region. The number of parasites is 1 - 8 copies. The morphology of the parasite, as a result of experimental studies, the average length of parasite females is 8.86 mm the anchor width is 2.35 mm. The morphological and morphometrical description are almost consistent with the description of *L. cyprinacea* in previous studies, which confirms that it is *L. cyprinacea*. Molecular identification 18S rRNA studies revealed a one nucleotide difference (99.8%) between the *Lernaea cyprinacea* species of this parasite and *L. cyprinacea* (KY435939) from the Genbank database.

INTRODUCTION

Parasitic infection is a common disease affecting commercial fish in ponds. Parasites inhibit the development of fish, resulting in reduced productivity and death **(Piasecki *et al.*, 2004; Bilal *et al.*, 2021)**.

The rapid development of aquaculture in Uzbekistan in recent years has increased the desire of fish farms to grow fish, and many new fish farms are being established. Prevention of the spread of infectious diseases in fish farming is the most important issue among farmers **(Daminov *et al.*, 2022)**. Most of the diseases that are encountered now are caused by the deterioration of the environment, causing the fish to become infected with diseases. In Uzbekistan, mainly warm water fish are grown, most of the fish are grown by polyculture method, which causes damage to several fish species with one parasite. Damage mainly affects to the growth and survival of fish **(Kurbanov, 2017)**.

In Uzbekistan, carp (*Cyprinus carpio*) is grown in fish farms as economically important species. However, these fish species are not free from parasitic diseases. Currently, the parasitic crustacean species *Lernaea cyprinacea* (Cyclopoida: Lernaeidae) is found in almost all regions of the Republic Uzbekistan **(Nomonov *et al.*, 2022)**. According to the data of these authors, in recent years, the degree of damage to the summer season has reached the highest rate of 55.8%, causing the most economic losses for farms.

The fact that this parasite is not studied well in Uzbekistan and the lack of scientific data makes it difficult to fight the disease. Currently, the remains of metamorphosed females are used to identify *Lernaea* species. A number of studies have shown that due to lerneosis, the shape of the handle (fixator) changes significantly **(Hua *et al.*, 2019)**. Consequently, more accurate identification invertebrates approaches have been developed using DNA sequencing technologies targeting the 18S, 28S ribosomal RNA, and cytochrome c oxidase subunit I mitochondrial DNA **(Song *et al.*, 2008; Pallavi *et al.*, 2017; Soares *et al.*, 2018; Madumarov *et al.*, 2021; Zhu *et al.*, 2021; Ikromov *et al.*, 2023)**. Although the above molecular markers are being used to identify *Lernaea* species, their availability is very limited.

This study aims to get information about *Lernaea* species that infect fish in Uzbekistan, in particular, in Tashkent and Fergana regions. Morphological and molecular identification studies were carried out to determine the taxonomic status of this parasite in the regions and provide general information about their watershed distribution.

MATERIALS AND METHODS

The researches were carried out on the carp fish grown in "Khorrot fish house" Yangiyol district and "TCT Fish cluster" Lower Chirchik district fishery farm Tashkent region and "Ibragimov Doston Fayz" fishery farm Fergana region of Uzbekistan (Fig. 1)

“Khorrot fish house	“TCT Fish cluster	Ibragimov Doston Fayz”
1) 41°03'22.0"N 69°00'12.9"E	1)40°53'57.2"N 68°47'34.1"E	1) 40°40'45.3"N 71°29'46.5"E
2) 41°03'18.3"N 69°00'13.0"E	2) 40°53'49.2"N 68°47'33.4"E	2) 40°40'44.7"N 71°29'50.1"E
3) 41°03'17.3"N 69°00'15.2"E	3) 40°53'41.2"N 68°47'31.7"E	3) 40°40'37.2"N 71°29'52.1"E

Fig. 1. General view of the research area. Biomaterial points collected from the area. ***** google earth site (https//earth. google. com) captured image

This research work was carried out from February 2022 to the second half of 2023 (from 9:00 am to 5:00 pm) using a net with a thickness of 5 mm and a mouth opening of

75x75 cm, weighing 115±1200 at different ages. A total of 240 fish samples of carp (*Caprinus carpio*) weighing 115±1200g were collected. The external body of the fish samples, including the head, left and right sides of the body, fins, around the eyes, abdomen and nose, were visually inspected. *Lernaea cyprinacea* parasites found in fish are carefully collected with tweezers, and the samples are fixed in 70% ethanol solution for detailed study. After collecting the samples, the fish were kept in a bath in 1% sodium chloride solution for 30 minutes and returned to the basin unharmed **(Bykhovskaya-Pavlovskaya, 1985)**. Learner body and anchor length were measured. All examined parasite samples were photographed, morphologically and morphometrically analyzed (Fig. 2). Specimens were visually described and photographed under a stereomicroscope microscope (Kern Optics OZL 456, Germany) equipped with a Spot insinght 2.0 Mp digital camera, and available literature was used for species identification **(Kabata, 1985; McCredden, 2016)**.

Dneasy Tissue Kit (Thermofisher.com) was used to extract genomic DNA from *Lernaea cyprinacea* species. For PCR, primers 18SF (5' CTGGTTGATYCTGCCAGT3' and 18SR (5'CTGAGATCCAACTAGGAGCTT3') of 18S rDNA used in molecular genetic identification of crustaceans were used **(Winnepenninckx *et al.*, 1995)**. Water for PCR 16.1 µl, 10x 2 µl of PCR buffer, 0.4 µl of dNTP, 2 µl of each primer, 0.4 µl of Taq polymerase were added, a total of 20 µl of mixture (mix) was prepared, and the following temperature regime was 92°C – 3 minutes; steps were performed at 92°C for 15 seconds, 55°C for 30 seconds, 72°C for 30 seconds (35 cycles), and 72°C for 10 minutes **(Soatov *et al.*, 2022)**.

The presence of DNA in PSR products was determined by electrophoresis on a 1.5% agarose gel at 120 V. A set of reagents produced by "Silex M" (Moscow, Russia) was used for DNA purification.

Sequencing PCR products ABI PRISM® BigDye Terminator v. 3.1 was performed using reagent kit, and the reaction products were recorded on an ABI PRISM 3100-Avant automatic sequencer (Moscow, Russia). Analysis of the received nucleotide sequence was carried out using Bioedit, Clustal W and DNAstarTM, PAUP4 special computer program.

Nucleotide sequences of the obtained Lernea shrimp were lake analysed using Genius prime software using DNA sequences from the international Genbank database (https://www.ncbi.nlm.nih.gov/), consensus sequences were calculated using Mega X computing software was calculated using Raw data from this program and complementary sequences from the GenBank database were compared using MAFFT v.7 online software and Clustal Omega 1.2.2 software using default settings. A phylogenetic tree was constructed using the maximum likelihood (ML) method with 500 bootstrap replicates **(Nei & Kumar, 2000)**. Evolutionary analyzes were performed in MEGA7 **(Kumar *et al.*, 2016)**. The 18S rRNA nucleotide sequences of lerneas in the Genbank

database and the cyclops species *Apocyclops royi* (AY626997) and *Macrocyclops albidus* (AJ746334) were used as outgroups (Table 1).

Table 1. Lernaeidae species used in the phylogenetic tree construction from the GenBank database (NCBI)

Parasites	GenBank no	Place
Lernaea cyprinacea_T	OR643672	Uzbekistan
Lernaea cyprinacea_F	OR643687	Uzbekistan
Lernaea cyprinacea	KY435939	South Africa
Apocyclops royi	AY626997	Great Britain
Macrocyclops albidus	AJ746334	Belgium

Morphological studies were carried out at the Laboratory of Ichthyopathology of the Fishery Scientific Research Institute, and molecular studies were performed at the Laboratory of Molecular Zoology of the Institute of Zoology of the Republic of Uzbekistan.

RESULTS

Parasite samples were found in all 3 researched areas. 76 out of 240 samples of the examined crucian carp were found to be infected with *Lernaea cyprinacea* (Table 2).

Table 2. The infection of the carp fish (*Caprinus carpio* L.) with *Lernaea cyprinacea* ectoparasite in the farm of Tashkent and Ferghana regions of Uzbekistan

The studied area	Number of fish examined	Infection of fish		Infection of intensity
		Number of fish affected	Prevalence, %	
Tashkent region **“Khorrot fish house”**	80	24	30	1-6
Tashkent region **“TCT Fish cluster”**	80	32	40	1-8
Fergana region **“Ibragimov Doston Fayz”**	80	20	25	1-4
Total:	240	76	31,6	1-8

Including in the fishery farm of "Khorrot fish house" Yangiyol district Tashkent region, infected 24 out of 80 fish samples, this is 30%, and 32 out of 80 fish samples in the fishery farm "TCT Fish cluster" in Quyi Chirchik district, which is 40%. At the "Ibragimov Doston Fayz" fishing farm in Fergana region, 20 out of 80 fish samples were infected, making up 25%. The highest level of infection at the farm "TCT Fish cluster" (40%) Tashkent region, and the lowest level of infection is 25% at the farm "Ibragimov Doston Fayz" in the Ferghana region (Table 2).

Morphology

In order to carry out a morphological study of parasites, 15 parasite samples were carefully studied morphologically. As a result of our experimental studies, the average length of parasite females is 7.23-13.40 mm, the anchor width is 1.45-6.35 mm. Morphology of *Lernaea* the swimming legs are located in the cephalothorax, the first swimming organ located in the middle of the body. The second and third leg segments are located in the trunk, respectively. The body is not divided into segments. A 1-2 mm long egg sac was found on the back of the female *L. cyprinacea.*

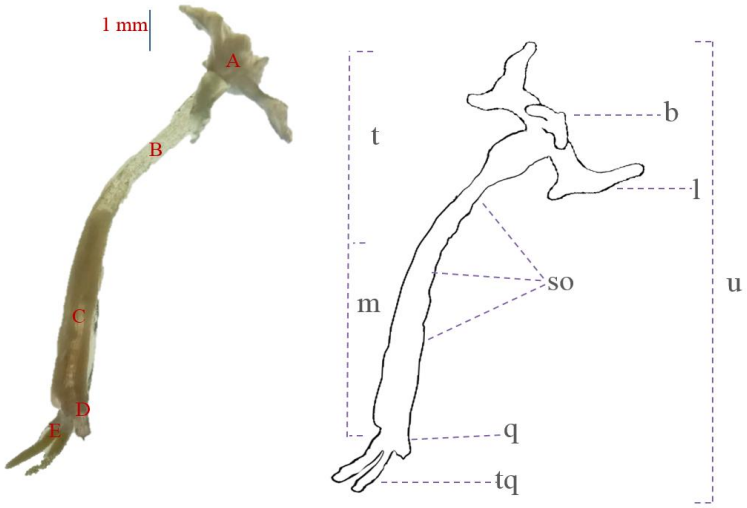

Fig. 2. Lateral view of a metamorphosed adult female of *Lernaea cyprinacea*. (left) (A) head, (B) coelothorax, (C) trunk, (D) abdomen, (E) eggs. Schematic morphology of *L. cyprinacea* (right) t= coelothorax; m= trunk b = head; l = anchor; so = swimming legs; tq = egg sac; q = abdomen; a = anal; u = total length

Table 3. Morphometric dimensions of female *L. cyprinacea* (n-10)

Symptoms of the parasite	***lim***	***M±m***	***Cv***	By **C.J.Hua (2019)**		
				lim	***M±m***	***Cv***
Body length	7.23–15.40	9.75 ± 1.44	20.35	6.67–12.3	8.86±1.71	19.30
Anchor width	1.45–6.35	3.20 ± 1.00	40.90	0.87–6.13	2.31±1.37	59.24
The dorsal part of the anchor	0.41–1.30	0.93 ± 0.15	41.27	0.26–1.74	0.90±0.39	42.91
Ventral part of the anchor	0.43–1.54	0.70 ± 0.25	38.47	0.31–1.01	0.55±0.21	37.39

Note: n - number of studied samples, lim - the variability limit of the symbol, M - arithmetic mean, m - mean arithmetic error, Cv - coefficient of variation. Confidence level R>0,05.

The morphology and morphometric description are almost consistent with the description of *L. cyprinacea* in previous studies, confirming that it is *L. cyprinacea* **(Gervasoni *et al.*, 2018; Hua *et al.*, 2019)**.

Molecular studies: According to the results of the molecular research, a nucleotide sequence of 620 base pairs belonging to the rDNA of 18S region of *L. cyprinacea* species was isolated. The sequences obtained from the samples were compared with the nucleotide sequence of *L. cyprinacea* (KY435939) and *Apocyclops royi* (AY626997) from another group of crustaceans in the international Genbank database (https://www.ncbi.nlm.nih.gov) (Fig. 3).

As can be seen from the table, 1 nucleotide difference was found between the samples of *L. cyprinacea* species collected from Tashkent and Fergana regions, and this difference was explained by the exchange of T-thymine in the *L.cyprinacea* - T sample and A-adenine nucleotides in the *L.cyprinacea* - F sample at the 71st nucleotide.

Lernaea cyprinacea species and *L. cyprinacea* (KY435939) obtained from the Genbank database differ by one nucleotide (99.8 %), in this 161 nucleotides A-adenine is present in *L. cyprinacea* sample, and in *L. cyprinacea* (KY435939) species, was found to be substituted on the basis of C-cytosine. From other crustaceans close to the *Lernea* species in the Genbank, it was noted that there were differences in 40 nucleotides between the *A.royi* (AY626997) and the *L. cyprinacea* sample (Fig. 3).

In particular, T-thymine in nucleotides 1, 148, 154, 178, and 210 in *L. cyprinacea*, C-cytosine in *A.royi*, 2, 38, 47, 90, 104, 179, 206, 212, 213 in *L. cyprinacea*. A-adenine to G-guanine in type A, A-adenine in type A 141, to C-cytosine in type A, T-thymine in nucleotides 97, 142, 143 in type A, to A-adenine in type A, G-guanine at nucleotides 91, 94, 134, 140, 155, 267, 407, 461 of *L. cyprinacea* species, A-adeninga of *A.royi* species, G-guanine at 239, 324, 375 nucleotides of *L. cyprinacea* species, *A.royi* type it was found that A-adenine in nucleotides 235, 253, 255 of *L. cyprinacea* was exchanged for T-thymine bases in T-thymine type A. It was found that it was exchanged.

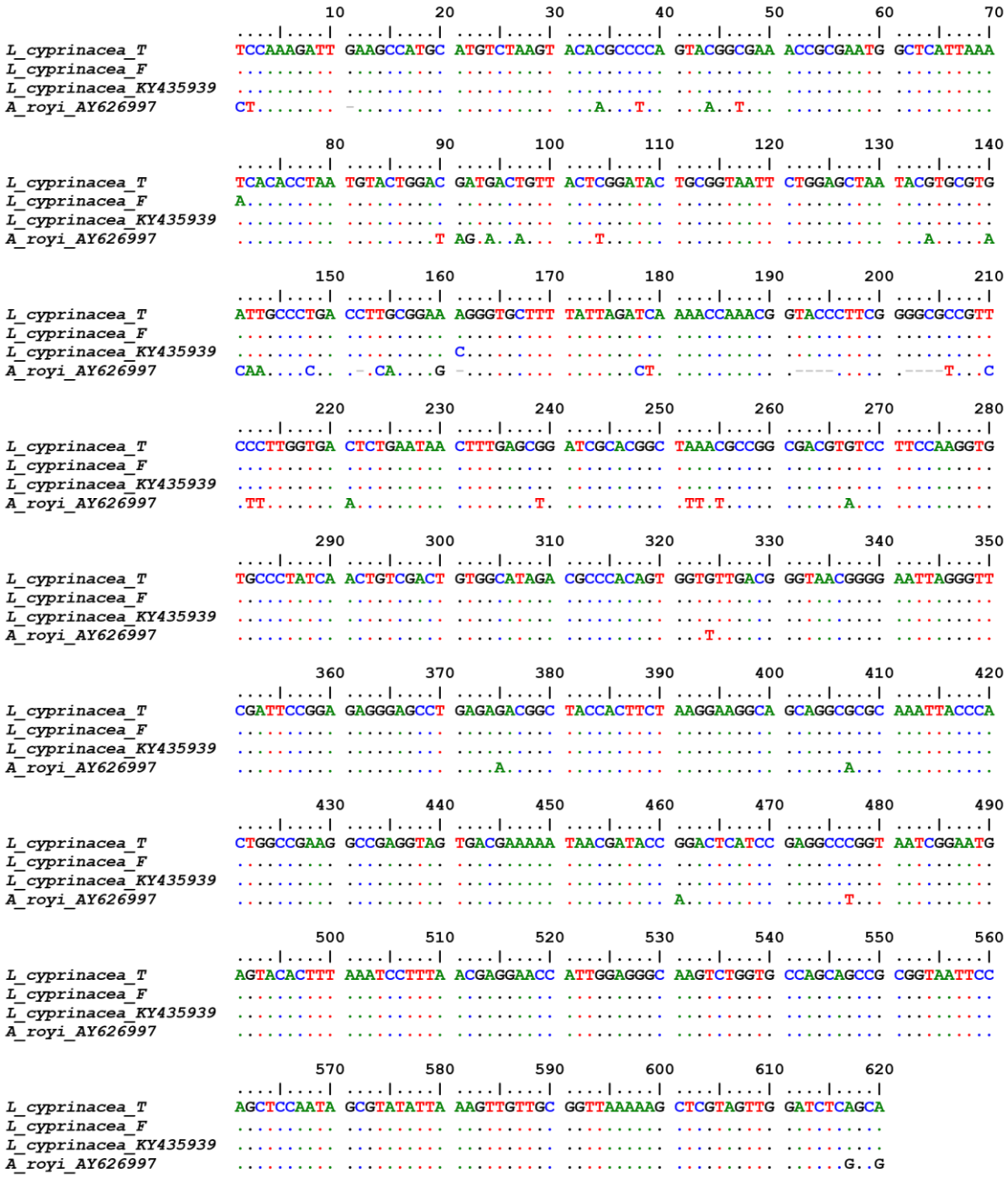

Fig. 3. Nucleotide sequence comparison of the 18S gene of the rDNA of species *Lernaea cyprinacea* and *Apocyclops royi.*

In the phylogenetic analysis, the phylogram based on the maximum likelihood (ML) algorithm gave the same results, that is, the monophyletic *Lernaea* clade and the outgroup *A. royi* and *Macrocyclops albidus* cyclops sequences were placed (Fig. 4).

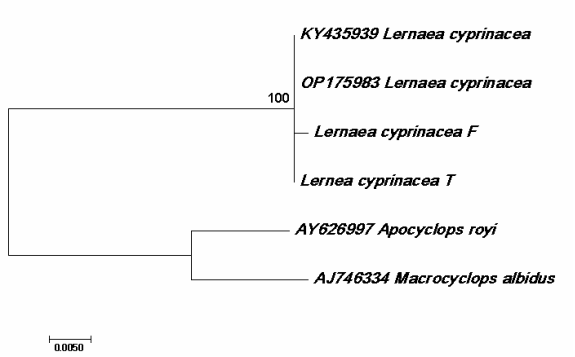

Fig. 4. Maximum-Likehood phylogeny of *Lernaea cyprinacea* 18S rRNA sequences of this study and sequences from the Genbank

DISCUSSION

According to **Kriswijayanti *et al.* (2019)**, if 1-5 *Lernaea* are detected in one fish, the level of *Lernaea* infestation is light, if 6-10 *Lernaea* are detected in one fish, it is moderate and more than 10 infected with *Lernaea*, it has been studied as severe damage. In our studies, *Lernea* numbers from 1 to 8 were found in infected fish. It was rated as moderately infected.

In *L. cyprinacea*, the body size of females is variable, and some of them are smaller than the research samples of some authors, and they show relatively large sizes. In particular, the samples measured in the research were smaller than the samples from Southern Europe, that is, the total length was 19-22.4 mm **(Ahnelt *et al.*, 2018)**. In Indonesia, the size is from 9.79 mm to 10.93 mm **(Nur *et al.*, 2022)**. Also, according to **Hua *et al.* (2019)**, it was found to be similar to the species distributed in China. The identified species of *L. cyprinacea* is **Gervasoni *et al.* (2018)** was found to be identical with *L. cyprinacea* cited for Argentina. In our study, the average length of female parasites was 8.68 mm, almost consistent with the description in previous studies.

CONCLUSION

According to the results of our research, samples of parasites were found in all 3 regions. It was found that 76 of the 240 samples of pollock fish were infected with *Lernaea cyprinacea.* The number of parasites is 1 - 8 copies. The highest level of damage was observed at the farm "TCT Fish cluster", and the lowest level of damage was at the farm "Ibragimov Doston Fayz" in Fergana region by 25%. As a result of our morphological experimental studies, the average length of parasite females is 8.86 mm, the anchor width is 2.35 mm. The morphology and morphometric description are almost consistent with the description of *L. cyprinacea* in previous studies, which confirms that it is *L. cyprinacea*. Molecular identification by 18S rRNA sumples of *Lernaea* species and *L. cyprinacea* (KY435939) obtained from the Genbank database differ by one nucleotide (99.8 %), in this 162 nucleotides A-adenine in *L. cyprinacea* sample, *L.*

cyprinacea (KY435939) species and it was found that it was substituted on the basis of C-cytosine.

Conflict of Interests

The authors declare that there is no conflict of interests.

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