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### ***Lernaea cruciata* (Crustacea: Copepoda) first report on infection to *Notopterus kapirot* in the Godavari river, Marathwada region, India**

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#### KEYWORDS

*Lernaea  
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#### A B S T R A C T

Teleosts from Nanded region of Marathwada, Maharashtra State in India were examined for the ecto-parasitic infection. *Notopterus kapirot* (Clupeidae) was found infected with *Lernaea cruciata* species of anchor worm. The infection was found Mostly to the lateral body surface. *Lernaea* infection was not found in Indian Major Carps including *Labeo rohita*, *Catla catal* and *Cirrihina mrigala*. It is first report of *Lernaea cruciata* infection to *Notopterus kapirot* in the world with the details of morphometric and meristic measurements of this anchor worm.

### Introduction

Fish is an important source of proteins for increasing population. More nutritious food supply is required to fulfill the requirement of animal proteins. Supply of healthy fish is important. Aquaculture industry is growing all over the world, with the main purpose of food & protein supply. The Aquaculture industry is known for its rank as the third biggest source of economic growth of the world. There are several endoparasites and ectoparasites found in fishes, amongst them the *Lernaea* is one of the dominant and commonly occurring ecto-parasitic copepod found all over the world from arctic, tropical and sub-tropical regions from Canada to Australia and USA to Korea, Japan.

The infection is found in India in fresh water fishes from all states and still it is an important challenge for the pathologists and fish farmers to get rid off from this tiny creature creating considerable damage to fish fauna. Under the uncontrolled conditions it cause direct killing in several thousands of economically important and commercial fish stock. Therefore it is essential to take review of the existence of species diversity, prevalence and intensity of infection in the fresh water fishery especially capture fishery in this region. There are no health hazards from *Lernaea* to human being because during scale removal and cleaning of the fish the *Lernaea* get

removed and the holdfast if remained in the host body get cooked as muscles and no any impact as a parasite on human body. But, it cause a great damage to fish as a food for human being before reaching it to human food dish which is great concern in solving high demand of proteinous natural animal food source in human diet.

It is difficult to estimate the number of Copepod parasites infecting fishes in the world but rough estimate by Geof et. al. (2008) and Kabata (1988) states that there may exists about 1,600 to 1,800 species distributed all over the world. A Crustacean Copepod *Lernaea* (Linnaeus, 1758) commonly called as anchor worm. It is an ecto-parasitic copepod on freshwater fishes reported from many parts of the world (Kabata,1979); (Lester and Hayward, 2006). It has remained a major ecto-parasitic copepod to fishes in fresh water aquaculture systems especially in to the integrated fish farming, in the Race way culture, Cage culture etc. It has wide distribution in almost all parts of the world except in arctic and Antarctic region. There are reports of the parasitism to fishes by this anchoring worm to wild and non-culturable fishes, cat fishes and other fishes. It shows infection to various parts of the host fish body. It was found to infect the dorsal, lateral, ventral, caudal, opercular region, branchial chamber, fin membrane, to upper jaws, lower jaw, chin region, in and near the eye orbit. It also infects the base of various fins and even the fin membranes, near the anal opening etc. The anterior parts of body of *Lernaea* spp. called holdfast organ remain embedded in to the host tissue while posterior elongated body remain free hanging (Photo Plate -A). The female of *Lernaea* has 10-20 mm. body size (kabata,1979), having anterior holdfast organ and posterior slender body with a pair of attached small, spindle form sac of eggs. The morphology of male *Lernaea* is not

known in detail and it die after copulation with female lernaea on host fish body (Grabda, 1963). The holdfast organ of the worm remain embedded into various parts of host body like skin, deep into body musculature on lateral or dorsal surface of body (Plate- A), in the eye orbit region, in the opercular region, branchial chamber, Fin membrane etc. The parasite has nine different stages of development in life cycle included three free swimming naupliua stages, five copepod stages and one adult stage. Female *Lernaea* spp. produce fertilized eggs after copulation and eggs remain stored in two/one egg sacs attached as a hanging structure with medium to short ovi-sac ducts in posterior extremity of adult female body (Grabda, 1963). *Lernaea* spp. are also ectoparasitic pathogens on cultured, wild and aquarium fishes (Shariff et. al., 1986; Lester and Hayward, 2006).

Amongst the 37 known species of *Lernaea* (Kabata, 1979) the species *L. cyprinacea* (Linnaeus, 1758) has been reported as cosmopolitan in distribution, common parasitic copepod infecting mainly carps in the culture and wild habitats; however Kabata (1988) is of opinion that, basically there are only two species of genus *Lernaea* and these are *L. cyprinacea* and *L. cruciata*. The species difference in *Lernaea* is mainly based on the morphological difference in the structure of holdfast organ, also the *Lernaea* spp. represents to some extent the host specificity. The thumb like projections, pegs, branchioles of main arms of holdfast organ are not sufficient to decide the characters for new species and therefore there is a controversy in number of new species of *Lernaea* (Kabata, 1988).

Based on these features, the species differences are being reported now days but by applying the modern approach to systematics, there may be synonymization of

several new species under the synonymization with *L. cyprinacea* or *L. cruciata*. Present investigation deals with the examination of several species of fresh water fishes from Godavari river basin including the habitats like river Godavari and its tributaries like Purna, Dudhna, Penganga, Kayadhu, Manjara, Aasna, Manyad, Lendi and Major reservoirs like Yelderi, Siddheshwar, Vishnupuri, Masoli, Borol, kandhar. In this report incidence of *Lernaea cruciata* species of anchor worm causing infection to *Notopterus kapirot* (Teleostomi : Clupeiformes, Notopteridae) from river Godavari in Nanded area of Marathwada region of Maharashtra state India was investigated. Also various morphometric variations in the *Lernaea* sp. *L. cruciata* collected mainly from *Notopterus kapirot* are explained in detail.

### **Materials and Methods**

Fish samples were examined for ectoparasitic infection from June- 2012 to June -2014 in Godavari river basin, Marathwada region, Maharashtra, India. The fish species mainly from family Cyprinidae, Clupeidae and Channidae were examined. From all the examined host fishes the anchor worm infection was observed in *Channa striatus* as most infected host fish species. The fish were examined by using magnifying lens initially on the site of study (fishing centers).

The morphometric, meristic characters were recorded, individuals were photographed for their external characters and host fish were identified up to species level using Froese & Pauly Fish Base (2014). The heavily infected host fish were fixed in 4% formalin and brought to the laboratory for further studies and preserved in Zoological museum, School of Life Sciences, S. R. T. M. University, Nanded. The *Lernaea* were

removed carefully from various body parts of host fish using forceps and fixed in 4 % hot formalin. About 50 ml. formalin was heated till air- bubbles comes out from the bottom of beaker and *Lernaea* collected in a petri-plate with little distilled water are placed into 4% hot formalin and stirred with glass rod. Till the cooling down of formalin in beaker, the *Lernaea* were kept and allowed to settle down. After 30 min. to 1 hr. the *Lernaea* were placed for preservation in 4 % formalin in eppendorfs and kept for one week duration for perfect fixation and further studies, for molecular studies the *Lernaea* sp. were fixed in 100% ethanol. after cleaning in distilled water. For long term preservation, the *Lernaea* sp. were post fixed into 70 % ethanol by removing from formalin fixation after 3 weeks. The stained micro-preparations were prepared by dehydrating the worms from 30, 50,70 % ethanol then stained in Carmine with acid hydrochloric (Aceto- Carmine) and again dehydrated from stain to 90 & 100 % ethanol each grade ethanol dehydration time was 5- 6 min. In the last step the worms were cleared in xylene and clove oil ( 1:1) for 1 Min, dipped and removed from xylene and embedded in Canada Balsam on slide and covered with rectangular cover glass.

The prepared slides were allowed to dry for 2-3 weeks by natural drying process or electric drying plate. After examination under compound binocular microscope (Carl-Zeiss) and microphotographs using Olympus Camera different morphological characters were recorded. In diagrams of morphological features were drawn using camera- lucida and measurement are in mm and  $\mu\text{m}$ . The specimen of *Lernaea* were identified by following the standard literature on *Lernaea* (Harding, 1950). The Permanent slides and whole specimen in 70 % and 100% ethanol of this well known anchor worm species are preserved in

parasite collection of Aquatic Parasitology and Fisheries Research Laboratory, Swami Ramanand Teerth Marathwada University, Nanded [(Specimen Acc. No. (ECT-C-L4) January, 2016].

Using binocular Microscope aided with 3 Mega Pixels magnification camera and using the selected Pixels of magnifications of the camera fixed to the microscope the different body parts measured for the minor details are as shown in above Table 1, (A), (B). The measurements are in mm scale and the calibration scale is as mentioned.

### **Results and Discussion**

After going through the literature and comparing the literature by following the keys to the identification of *Lernaea* (Harding, 1950 and Kabata, 1984) the present specimen come close to the *Lernaea* species *Lernaea cruciata* hence it has been confirmed that the present species collected from host fish is *Lernaea cruciata* due to single simple distinguishing feature that it has Y- shaped anchor organ.

First time in the present study various body parts of the parasite i. e. accurate total length, length of the arms of anchor organ, diameter of trunk region, length and diameter of ovi-sac was measured, this is the new reports as redescription of this species. Similarly it is the first report of occurrence of *Lernaea* on the fresh water knife fish from Maharashtra State, India.

key to the *Lernaea* species identification [Harding (1950) and Kabata (1998)]

Anchor organ or holdfast organ to the anterior side having two pairs of branches: One large T-shaped branch at the lateral position and another small, simple pair, usually short and thin and situated mostly

interiorly or posterior to cephalo-thorax. *Lernaea cyprinacea* (Linnaeus, 1758)

Anterior anchor or holdfast organ having single pair of Y-shaped branches *Lernaea cruciata* (Le, Sueur, 1824).

Morphometric and meristic details of the anchor worm *Lernaea* collected are as given in following table and figures.

### **Name of the parasite**

*Lernaea cruciata* (R. D.)

### **Technical details of museum record**

Acc. No. ECT-CL-7. Figure Magnification PXLs (121.07 = 0.964 mm)

### **Name of the host Fish**

*Notopterus kaporat*

**Parasite Collected from :** Lateral body surface of host fish.

**Locality of Collection:** Godavari River, Vishnupuri, Nanded, MS, India.

**Date of Collection:** April 2<sup>nd</sup>, 2015.

The specimen was measured using binocular Microscope aided with 3 Mega Pixels magnification camera 121.07 PXLs = 0.964 mm was magnifications Pixels selected in the camera fixed to the microscope the different body parts measured for the minor details are as shown in above table. The measurements are in mm scale and the calibration scale is as mentioned above. The mm scale bar is as indicated in the figure for 1 mm. The measurements of the specimen are as in Table given above. After going through the literature and comparing the literature by following the keys to the

identification of *Lernaea* (Harding, 1950 and Kabata, 1984) the present specimen come close to the *Lernaea* species *Lernaea cruciata* due to Anchor with Y-shaped arms. The present specimen comes close to *Lernaea cruciata* (Harding, 1950) due to distinguishing feature that it has Y- shaped anchor organ. It has a special stalked spindle form egg sac (Photo Plate 1-A- labeled-3) having length 0.964 mm and length of duct of the ovi-sac 0.339 mm. After going through the literature and comparing the

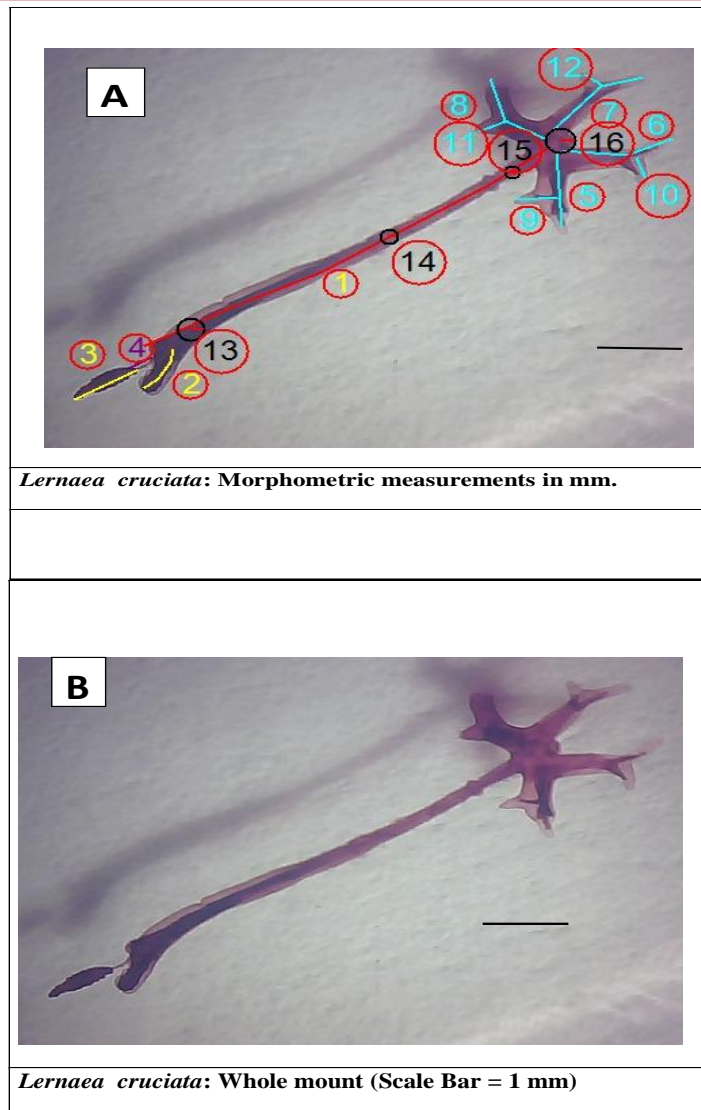
literature by following the keys to the identification of *Lernaea* (Yamagtti, 1963; Harding, 1950 and Kabata, 1984) the present specimen come close to the *Lernaea* species *Lernaea cruciata* hence it has been confirmed that the present species collected from host fish is *Lernaea cruciata* due to single simple distinguishing feature that it has Y; shaped anchor organ, In addition the various body parts measured in mm is an additional data for redescriptionof this species.

**Table.1A** Details of Morphometric Measurements of *Lernaea cruciata*

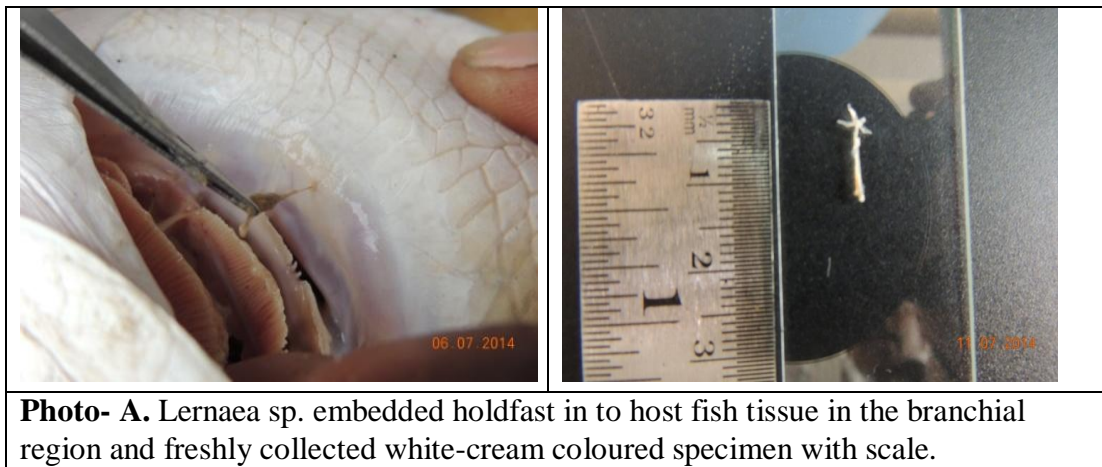
<b>Particulars of measurements of body</b>	<b>Unit</b>	<b>Length</b>
Length of body up to the legs (1)	mm	6.195
Length of Post-genital prominence (2)	mm	0.768
Length of spindle form Ovisac (3)	mm	0.964
Length of Ovi-sac duct (4)	mm	0.339
Length of arm of an anchor organ (5)	mm	1.196
Length of arm of an anchor organ (6)	mm	1.518
Length of arm of an anchor organ (7)	mm	1.518
Length of arm of an anchor organ (8)	mm	1.285
Length of arm extension of an anchor organ (9)	mm	0.553
Length of arm extension of an anchor organ (10)	mm	0.375
Length of arm extension of an anchor organ (11)	mm	0.428
Length of arm extension of an anchor organ (12)	mm	0.321

**Table.1B** Measurements of Diameter of Body Parts

<b>Particulars of measures</b>	<b>Unit</b>	<b>Radius</b>	<b>Diameter</b>	<b>Perimeter</b>	<b>Area</b>
Measurements of posterior region of trunk (13)	mm	0.179	0.357	1.122	0.100
Measurements of middle region of trunk (14)	mm	0.107	0.214	0.673	0.036
Measurements of neck region of trunk 15	mm	0.089	0.179	0.561	0.025
Measurements of cephalo-thorax (16)	mm	0.196	0.393	1.234	0.121



**Photo Plate.1** *Lernaea Crucciata* (A- Whole Mount Body Measurements, B- Whole Mount), (Length Of Scale Bar = 1 Mm)



Using binocular Microscope aided with 3 Mega Pixels magnification camera and using the selected Pixels of magnifications of the camera fixed to the microscope the different body parts measured for the minor details are as shown in above table. The measurements are in mm scale and the calibration scale is as mentioned above. First time in the present study various body parts of the parasite i. e. accurate total length, length of the arms of anchor organ, diameter of trunk region, length and diameter of ovi-sac was measured these are the new reports as redescription of this species. Similarly it is the first report of occurrence of this species of *Lernaea* on the fresh water fish *Notopterus kapirot* from Maharashtra State, India.

Due to deep burrowing of anchor organ of this specimen in to the host body musculature hardly it was able to collect the intact specimen, one arm anterior point get broken in the collection process. A kind of internal chitinous supporting structures also appear in the photo plate with thin covering of smooth tissue. It was first time this set of specimen was treated with the dilute solution of KOH (10 %) heated to 25-30<sup>0</sup> C on gas flame for 4-5 minutes. For the internal organ studies instead of staining directly the specimen may be treated with glycerin after treatment with KOH and mounted as W.M. on the slide and covered with cover glass sealed by nail-paint border, but we mounted the specimen in Canada Balsam.

In earlier studies on *Lernaea sp.* infection Mirza (2015) examined 3520 specimen of ornamental fish and found 5.3 % infection in 186 number of fishes. The infection of *Lernaea cyprinacea* was found during summer season where as in the present investigation *L. cruciata* was found infecting *Notopterus kapirot*; The intensity of infection was % infection 44.58 %. From the

observed 10 habitats highest % of infected fishes were from the reservoirs in the region.

Aline Angelina (2013) investigated the occurrence of *L. cyprinacea* in the fishes from Brazilian river and found that the infection was not to organ specific in 60 host fishes examined that belongs to native species *Skeindachneria insulpta*. In the present study the infection of *Lernaea species* was found region specific on the host fish body. Maximum infection was found on the lateral surface of body. Raisy et. al. (2012) examined Cyprinid fishes from Chogakhor lagoon due to outbreak of *Lernaea cyprinacea*. Prevalance and intensity of infection was recorded and correlated with age, total body weight and total length of body. Seasonal variation and population dynamics of *L. cyprinacea* was recorded, In the present study remarkably it was observed that there was no infection on the cyprinid fishes examined from the natural habitats which are sites of capture fishery of the region. In another study on *Lernaea* infection to fish stock at a private fish farm 'Zaidi Fish Farm' in Multan, Pakistan Kanwal et. al. (2012) carried out a study on *Lernaeid* parasites of *Catla catla* and found 32 % prevalence of *L. cyprinacea* in body weight group 50-100 gm but 0.0 % prevalence of *Lernaea* on large body size group such as 2,900 gm and more. Similar results were obtained in the present study too. Another author in recent years 2012 found *Lernaea cyprinacea* was most prevalent parasite on Indian Major Carps and Chinese Carps during the examination of *Lernaea* infection in experimental ponds of Punjab University, Lahore, Pakistan. In this study with increase in length and body weight the rate of infection was decreased. Iqbal et. al. (2012) from Pakistan Lahore also found same results in IMC and found 87-100 % infestation but they found mainly *L. cyprinacea*. Tasawar et. al. (2009) recorded four species of *Lernaea* from Grass

carp from Multan Pakistan where as in the present study there was no grass carp in the catch composition from wild resources like river, streams and reservoirs of Godavari river basin in the selected study area. In the market surveys also no Grass Carp was found. Amina (2009) examined parasitic infection by *Lernaea* on the Gold Fish (*C. auratus*) in some aquaria from Egypt and noted variation in the morphology and color, length, size of *Lernaea* collected; In the present study morphometric measurements were conducted for the identification first time we tried to measure each part of the *Lernaea cruciata* species collected, such measurements of the cephalo-thoracic region, egg sac, post and pre genital prominence and other parts mentioned in the Table 1 (A, (B). These measurement details were not found in the earlier reports.

In another investigation Tasawar et. al. (2009) examined 200 *Catla catla* from private farm at Multan Pakistan and reported the infection of female *Lernaea* species. In this study also Tasawar (2009) found reduced % of infection of *Lernaea* species in 1 % in larger fish samples amongst the observed 200-400 gm. Bote (2008) collected fish samples from very large geographical area of about 25,000 Km<sup>2</sup> at 25 sample stations from large rivers in France and found 4.34 % prevalence of *L. cyprinacea* on migratory fish species Alis shad, it has not been reported specific to season and body parts of the host in the selected host; where as in the present study the infection was found in the capture fishery in *Notopterus kapirot* which show local migration within the habitat. Maxwell et. al. (2008) investigated on *Lernaea* outbreak in a Wild Life Reserve Malilangwe, Zimbabwe and found *Lernaea cyprinacea* on the fish species from three families namely Cyprinidae, Cichlidae and Claridae. The infection was on the lateral and caudal region mainly. In the present study the

infection was found on almost all body parts of the examined parasitized host fishes but maximum on lateral side of the body. *Notopterus kapirot* has laterally compressed almost flat body surface hence there might be sufficient space for the attachment of this *Lernaea* species in this host fish.

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