Turkiye Parazitol Derg 2022;46(3):213-8
DOI: 10.4274/tpd.galenos.2022.41736

Identification of Larval *Eustrongylides* (Nematoda: Dioctophymatoidea) sp. from *Channa punctata* Bloch, 1793 by Morphological and Molecular Techniques

Channa punctata Bloch'tan Larva Eustrongylides (Nematoda: Dioctophymatoidea) sp.'nin Morfolojik ve Moleküler Tekniklerle Tanımlanması, 1793

Ivy Kundu[1], Dipak Ranjan Mandal[2]

[1]Barasat Government College, Department of Zoology, West Bengal, India
[2]South Asian Forum for Environment, West Bengal, India

Cite this article as: Kundu I, Mandal DR. Identification of Larval *Eustrongylides* (Nematoda: Dioctophymatoidea) sp. from *Channa punctata* Bloch, 1793 by Morphological and Molecular Techniques. Turkiye Parazitol Derg 2022;46(3):213-8.

ABSTRACT

Objective: This study aimed to identify the larval form of *Eustrongylides* sp. isolated from the visceral organs of *Channa punctata* (Bloch, 1793) using morphological and molecular methods.
Methods: Fishes were collected from fish farms in Nadia and North 24 Paraganas for the collection of nematodes. The visceral organs were dissected and kept in 0.67% normal saline. Nematodes collected from the abdominal regions and visceral organs for light microscopy study were fixed in 70% ethanol. Morphological features were studied by placing the nematodes in lactophenol. The specimens were later preserved in 70% ethanol containing 5% glycerine. Specimens processed for scanning electron microscopy were fixed in 2.5% glutaraldehyde and postfixed in 1% osmium tetraoxide. Proper identification was done by using standard methology. Molecular studies were performed for the *18S rRNA, ITS1, 5.8S rRNA, ITS2* and *28S rRNA* gene fragments using polymerase chain reaction amplification, sequencing and phylogenetic analysis.
Results: The morphological characteristics of nematodes were described with the help of light and scanning electron microscopy. Additional features not described earlier like dimensions and shape of the cephalic papillae, absence of somatic papillae, presence of caudal papillae, were identified for the first time. Moreover, molecular studies with ITS regions further confirmed the identification of the nematode.
Conclusion: Thus the use of morphotaxonomy along with molecular techniques would help in proper identification of *Eustrongylides* sp infecting edible fish. Studies on the nematode would help toexplore the intermediate as well as paratenic hosts of the parasite. Data in this regard would contribute significantly to the fish database in regard to parasites infesting edible fishes.
Keywords: *Channa punctata, Eustrongylides* sp., scanning electron microscopy, taxonomy, molecular identification

ÖZ

Amaç: Bu çalışmanın amacı, *Channa punktata*'nın (Bloch, 1793) visseral organlarından izole edilen Eustrongylides sp.'nin larva formunun morfolojik ve moleküler yöntemlerle belirlenmesidir.
Yöntemler: Balıklar, nematodların toplanması için Nadia ve Kuzey 24 Paraganas'taki balık çiftliklerinden toplandı. İç organlar diseke edildi ve %0,67 normal salin içinde tutuldu. Işık mikroskobu çalışması için karın bölgelerinden ve iç organlardan toplanan nematodlar, %70 etanol içinde sabitlendi. Nematodlar laktofenol içerisine yerleştirilerek morfolojik özellikleri incelendi. Numuneler daha sonra %5 gliserin içeren %70 etanol içinde muhafaza edildi. Taramalı elektron mikroskobu için işlenen numuneler %2,5 glutaraldehit içinde ve daha sonar %1 osmiyum tetraoksit içinde sabitlendi. Standart metodoloji kullanılarak uygun tanımlama yapıldı. *18S rRNA, ITS1, 5.8S rRNA, ITS2* ve 28S rRNA gen fragmanı için polimeraz zincir reaksiyon amplifikasyonu ve dizileme kullanılarak moleküler çalışmalar ve filogenetik analiz yapıldı.

Received/Geliş Tarihi: 29.04.2021 **Accepted/Kabul Tarihi:** 24.06.2022

Address for Correspondence/Yazar Adresi: Ivy Kundu, Barasat Government College, Department of Zoology, West Bengal, India
Phone/Tel: +09433994105 **E-mail/E-Posta:** ivy_kundu@rediffmail.com **ORCID ID:** orcid.org/0000-0003-3467-4620

Türkiye PARAZİTOLOJİ Dergisi

Bulgular: Nematodların morfolojik özellikleri ışık ve taramalı elektron mikroskobu yardımıyla yeniden tanımlanmıştır. Sefalik papillaların boyutları ve şekli, somatik papillaların yokluğu, kaudal papillaların varlığı gibi daha önce tanımlanmayan ek özellikler ilk kez tanımlanmıştır. Ayrıca, ITS bölgeleriyle yapılan moleküler çalışmalar, nematodun tanımını daha da doğrulamıştır.
Sonuç: Moleküler tekniklerle birlikte morfotaksonominin kullanılması, yenilebilir balıkları enfekte eden *Eustrongylides* sp.'nin doğru tanımlanmasına yardımcı olacaktır. Nematod üzerinde yapılan çalışmalar, parazitin ara ve paratenik konaklarının araştırılmasına yardımcı olacaktır. Bu bağlamdaki veriler, yenilebilir balıkları istila eden parazitlerle ilgili olarak balık veritabanına önemli ölçüde katkıda bulunacaktır.
Anahtar Kelimeler: *Channa punctata, Eustrongylides* sp., taramalı elektron mikroskopisi, taksonomi, moleküler kimlik

INTRODUCTION

Fishes diseases are prevalent as they act as host in the life cycle of various organisms which severely affects the economic value of the marketable fishes. This is important particularly for those fishes which are consumed raw or smoken (1). In aquaculture practices parasitic infection like bacteria, protozoa, helminths, fungus and viruses causes huge financial deprivation and diminishes the biodiversity of indigenous species. Platyhelminths and Nemathelminths are two phyla under which helminth parasites are included (2). Intermediate stages of nematodes, cestodes and trematodes affects internal visceral organs of fishes, while monogeneans being ectoparasites infest on gills and skin (2). In the life cycle of endoparasitic helminthes the parasite passes through intermediate stages and finally develops into an adult in higher vertebrates like piscivorous, birds, mammals, man that feed on the fish. The larval stage shows adaptation that enables them to survive and reach adult stage (3). Dynamics of aquatic system is better evaluated and understood due to the study of life cycle of helminth which involves more than one intermediate hosts (4). Mollucs and copepods also act as intermediate host in the development of various helminth parasites.

Channa punctata Bloch, 1793 commonly known as snake headed fish belongs to family Channidae and is well known for its nutritional value. The fresh water fish, being a prolific breeder is commercially reared and accords significantly to aquaculture sector. Taxonomy of nematodes were studied by researchers (5-7) collected from fishes. Information on the database of helminth parasites infecting fishes in different districts of West Bengal is inadequate with incomplete representation. Hence the present study was conducted to provide a comprehensive data of nematode parasite for proper characterization infecting economically important fishes like *Channa punctata*.

METHODS

Collection of Fish Samples and Parasites

The host fishes collected from fish farms of various districts of West Bengal like Nadia (22° 59' 0" N, 88° 29' 0" E) and North 24 Paraganas (22.7228° N, 88.4806° E) during the period of December 2017-January 2020 and were brought to the parasitology laboratory for examination. As per CPCSEA instruction's protocol for experimentation on fishes, does not require approval from ethical committee. Nematodes collected were first washed in normal saline, then fixed in 70% ethanol. Later they were examined in lactophenol for light microscopical examination and morphological observation. All collected specimens were then preserved in 70% ethanol and 5% glycerine. Identification of the parasites were done using standard methology using identification keys (8-10).

Sample Preparation for Scanning Electron Microscopic Study

The helminth parasites were fixed in 2.5% glutaraldehyde solution (pH 7.4) in 0.1 M sodium cacodylate buffer at 4 °C, postfixed in 1% osmium tetraoxide. Dehydrated through varying alcoholic concentrations followed by wash with absolute alcohol and amyl acetate mixture of different ratios (3:1, 2:2 and 1:3) and finally in 100% amyl acetate. Specimens were then coated with gold using a coater (Quorum Q 150 TES) by placing them on stubs with double adhesive tape. Coated samples were examined with a high-resolution scanning electron microscope (Zeiss EVO-MA 10 Germany) operating at accelerating voltages of 15KV.

Illustrations and Measurement

Measurements were taken with the use of stage and an ocular micrometer while figures were drawn by using camera lucida. Light microscopic photographs of parasites were taken by camera-mounted microscope (Olympus CX41).

Statistical Analysis

Statistical analysis was conducted using SPSS software. All measurements are given in millimeters if not stated otherwise. Parasite specimens (holotype and paratypes) were deposited and preserved in the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani in West Bengal, India.

Genotypic Characterization

The identified parasites specimens were further confirmed by modern molecular technique such as 18S rDNA, ITS1, 5.8S rRNA, ITS2 and 28S rRNA analysis. DNA was isolated using the genomic DNA isolation kit according to the instructions of the manufacture (Bangalore Genei and Eurofins).

Polymerase Chain Reaction (PCR) Amplification and Sequencing

In case of *Eustrongylides* sp. the 18S rRNA, ITS1, 5.8S rRNA, ITS2 and 28S rRNA gene fragment rDNA sequences was amplified by PCR. The primer sequences forward 5'GGAAGTAAAAGTCGTAACAAGG 3' and reverse 5' CCGTCTTGAAACACGGACC 3' were used. The PCR mixture consisted of 1X assay buffer (Mg_2^+ free), 1 µL of dNTP mixtures (2.5 mM each), 100 ng of each primer (forward and reverse), 200 ng of template DNA and Taq DNA polymerase (3 U/µL) in a final volume of 50 µL. PCR was performed in a thermal cycler using an initial denaturation at 94 °C for five minutes, followed by thirty-five cycles at 94 °C for thirty seconds, 56 °C for thirty seconds and 72 °C for one minute and final extension at 72 °C for 10 minutes. Electrophoresis of the PCR products (10 mL) lead to its separation on 1.0% agarose gel for 1 h in 1X TBE (Tris-Boric acid-EDTA) buffer. Amplicons were detached from gel and purified using a PCR purification kit eluted in Tris-HCl (10 mM, pH 8.5) prior to sequencing.

Sequencing and Phylogenetic Analysis

In case of *Eustrongylides* sp. amplification of the rDNA genes (1090b bp) were performed by automated sequencer to obtain the sequence information. Clustal X mega software was used for editing the sequences and from the National Center for Biotechnology Information database, BLAST search was done. The knowledge of the nearest neighbors of the amplified sequence was used in homology studies.

RESULTS AND DISCUSSION

Macroscopic Observation

Nematodes were recovered from the abdominal cavity, visceral organs, musculature, and have been identified as *Eustrongylides* sp. based on larval morphological features. The anterior one-third of the larval body was yellowish to red, while the rest was bright red in colour with visible cuticle stratification which was retained by the larvae description of nematode parasite, *Eustrongylides* sp.

Site of infection: Liver, intestine, abdominal cavity, 33.33% prevalence.

Specimen bearing numbers PR/IK/N-09, PR/IK/N-12 and PR/IK/N-15 have been deposited in Parasitology Laboratory, Department of Zoology, University of Kalyani, West Bengal, India

Identifying characters: (24 nematodes, Figure 1, 2).

Light Microscopic Study

Body of smaller larvae whitish while larger larvae red-brown in colour. Cuticle coarsely striated but without spines (Figure 1a). Head not particularly swollen (Figure 1a). Nerve ring arises from the anterior end. Buccal cavity followed by long oesophagus long without any particular swelling (Figure 1a).

Figure 1. Light microscopic structure of *Eustrongylides* sp. a) Anterior portion of nematode *Eustrongylides* sp. showing the oesophagus (os), spine shaped papillae (pa) and striations (st). scale bar - 1 mm, b) Caudal (ca) portion of nematode Eustrongylides sp. scale bar -1 mm

Mouth simple with twelve papillae arranged in two circles, always two lateral and four submedian in each circle (Figure 1a). Papillae of the internal circle have a long, tapering, spine like central process while papillae of the external circle were low, dome shaped with wide bases. Many caudal papillae were present at the posterior end which were smooth and oblate. Male bursa closed, bell shaped without rays. Female posterior end stumpy (Figure 1b). Anus terminal and the position of vulva lies very close to the anus. Adults usually detected in the glands of fore stomach of aquatic birds and larva in fishes.

Scanning Electron Microscopic Structure

Scanning electron microscopic images of *Eustrongylides* sp. helped further to elucidate the taxonomic features of the isolated parasite. SEM studies performed showed that the cephalic extremity was conical having twelve labial papillae placed in two rings having six papillae each. Each circle consisted of two lateral, two subventral and two subdorsal papillae (Figure 2a). Presence of sensilla above the papillae was observed (Figure 2a). Amidst two outer subventral papillae, horizontal flat ventral papillae was present (Figure 2a). Inner and outer papillae were of similar size, while the inner papillae had tapered base with spine-like apices (Figure 2b). Cephalic and labial papillae bears a distinct ring and the border being marked by a cuticular depression (Figure 2b, c). Outer papillae had broad bases with dome shaped apices (Figure 2c). Nematode bears transverse cuticular striations with many flat caudal papillae at tail end (Figure 2d).

Molecular Studies

ITS-1, 5.8S, and ITS-2 regions of the nuclear rDNA region were amplified using PCR primers and the lengths of the PCR products was found to be 1090 bp. The analysis further shows that the sequence contains 320 adenine bases, 185 cytosine bases, 297 guanine bases and 288 thymine bases. Thus GC content of the ribosomal RNA molecule has 44.2% and 55.8% AT content. The rRNA gene amplified resulted in a 1090-bp fragment (Figure 3). The sequence was submitted in Genbank under the accession number KJ458967. The consensus sequence analysed by BLAST search gave a 100% identity with *Eustrongylides* sp. 16 XF-2009 (GQ215514), *Eustrongylides* sp. 35 XF-2009 (GQ215533). 99% identity with *Eustrongylides* sp. 74 XF-2009 (GQ215572), *Eustrongylides* sp. 54 XF-2009 (GQ215552), *Eustrongylides* sp. 61 XF-2009 (GQ215559) and *Eustrongylides* sp. 42 XF-2009 (GQ215540). 98.0% with *Eustrongylides* sp. 4 XF-2009 (GQ215502). 97% similarity with *Eustrongylides* sp. 53 XF-2009 (GQ215551), *Eustrongylides* sp.79XF-2009 (GQ215577) and *Eustrongylides excisus* isolate 319/17B12 (GQ215528). Phylogenetic tree constructed using neighbor joining method shows the studied sequence forms a clade with other closely related *Eustrongylides* sp. with bootstrap values ranging from 77-95% (Figure 4). The alignment of the rRNA showed few differences (using Kimura 2 parameters) between the studied sequences, with distances ranging from 0.001% to 0.037% (Table 1).

Fishes acts as either the second intermediate or paratenic hosts (other paratenic hosts may be amphibians and reptiles) harbouring the third and fourth-stage *Eustrongylides* larvae while the definitive hosts are various fish-eating birds. Taxonomic studies depicted that the length, width and length of oesophagus of the larvae were found to be greater than that studied by Moravec et al. (11), Xiong et al. (12), Novakov et

al. (13) however the number, arrangement and shape of the cephalic papillae as well as transverse cuticular striations were found to be similar. The morphometrics of the parasite obtained however correspond to the data provided by Llchtenfels and Pilitt (14). Moravec et al. (11) reported presence of *Eustrongylides* sp. athough there was no mention of the stage and neither identification upto species level was done. Comparison of the findings of Xiong et al. (12), and Llchtenfels and Pilitt (14), it can be concluded that present nematode appears to be the fourth stage of development as the third stage lacks the round base with cuticular depression. A comparative measurement account of the genus is given in Table 2.

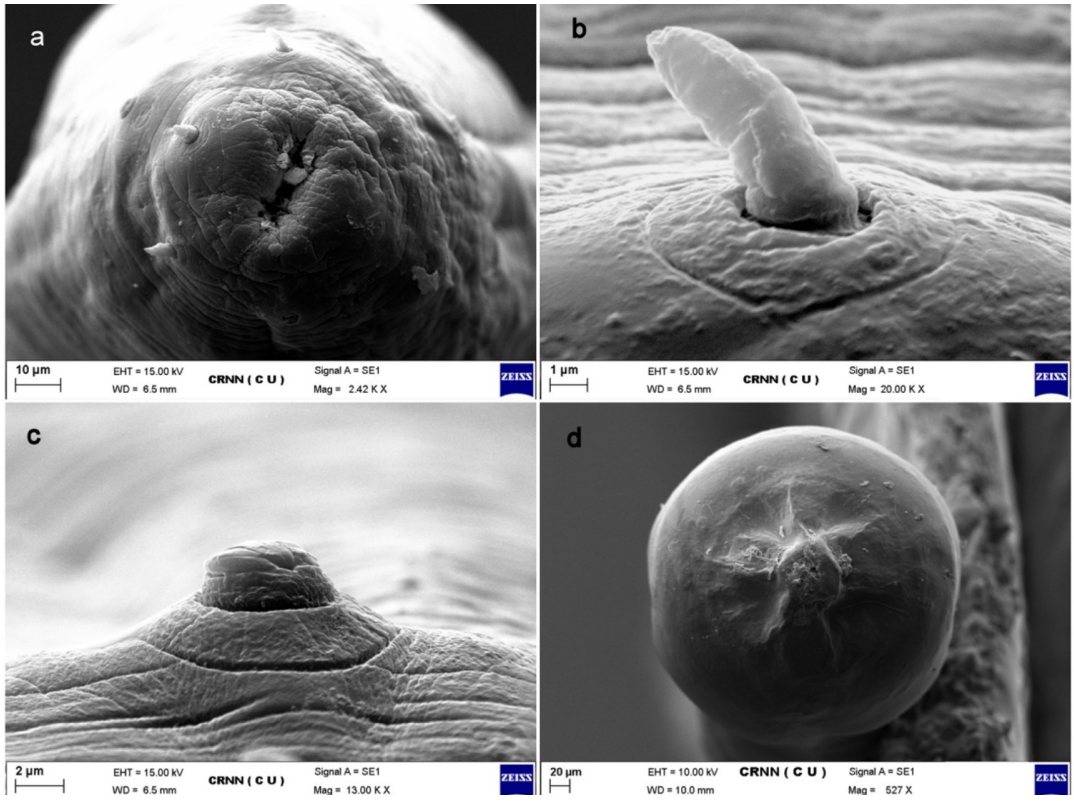

Figure 2. Scanning electron microscopic structures of *Eustrongylides* sp. a) Anterior portion of nematode *Eustrongylides* sp. showing the mouth, arrangement of domeshaped and spine shaped papillae (pa) in two circles. Cuticular striations prominent, b) Enlarged portion of spine shaped papillae (pa), c) Enlarged portion of dome shaped papillae (pa), d) Papillae (pa) present in the caudal (ca) region of *Eustrongylides* sp.

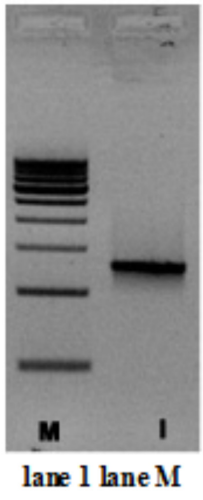

Figure 3. Nucleotide composition of *Eustrongylides* sp. isolated from Channa punctata (KJ458967) and agarose gel electrophoresis showing the PCR amplified product in lane 1 and StepUpTM 500bp DNA ladder in lane M

PCR: Polymerase chain reaction

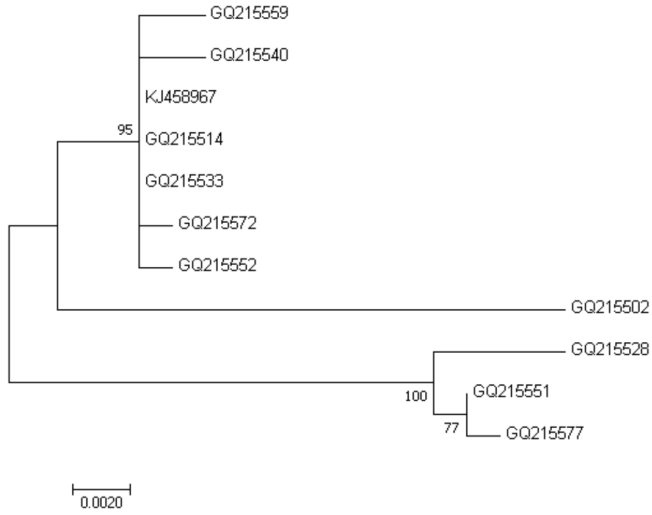

Figure 4. Phylogenetic tree constructed using neighbor joining method of *Eustrongylides* sp. using ten similar sequences along with representative sequences KJ458967. The numbers associated with the branches represent the bootstrap values

In the present study the SEM showed the details of the arrangement, structure and shape of the inner and outer circle papillae in the cephalic end which corroborates with the study of Xiong et al. (12) and Mazzone et al. (15). Somatic papillae was not detected in the larval stage of the parasite during in the present study which was similar to the observation of Xiong et al. (12) however Mazzone et al. (15) and Gupta (16) have reported the presence the papillae arranged in one row in each lateral field from cephalic to anal end. Sensilla was detected above the cephalic papillae which is similar to the reports of Gupta (16). Many smooth and oblate caudal papillae were observed in the present study, were also reported by Xiong et al. (12) although Gupta (16) did not report any such observations. Differentiation of both third- and fourth stage of *Eustrongylides* sp. collected from fishes were done by both Crites (17); Llchtenfels and Pilitt (14) by scanning electron microscopy which further confirmed the fourth stage of development of the larva isolated in the current study.

Xiong et al. (12) identified the fourth stage larvae of *E. ignotus* and differentiated it from *E. tubifex* and *E. excisus*, Čabrilo et al. (18) first reported *Eustrongylides excisus* Jägerskiöld, 1909 larvae in pike-perch *Sander lucioperca* by light microscopic study only, Gupta (16) reported *E. excises* from *Channa punctatus* by morphological characters although no molecular characterization neither transmission experiments was performed. Though Measures (19,20) had the opinion that species identification of *Eustrongylides* larvae from fishes is very difficult, unless verified by feeding experiments to bird definitive hosts as interspecific morphological differences are found only in adults.

Further more molecular procedures have played an important role in identification of parasites McManus and Bowles (21). BLAST analysis of the sequence from studied parasite shows high genetic similarity with *Eustrongylides* sp. compared with similar sequences in Genbank. The analysis carried out on the molecular sequence fragment region confirmed that parasites belong to the species *Eustrongylides* sp. display is 100% identity in the similar gene fragment region as the same nematode was collected by Xiong et al. (22) from different fish species. Thus along with morphometric studies, molecular data confirmed the isolated parasite to be *Eustrongylides* sp. In phylogenetic tree, high bootstrap value of the molecular sequence KJ458967 accounts for the maximum likelihood and the clustering pattern with that of *Eustrongylides* sp. clade. The sequences collected from various fishes worldwide shows high level of similarity which suggests that *Eustrongylides* sp. could form communities of the same species. Xiong et al. (22) reported that phylogenetic analysis of mitochondrial cytochrome oxidase c subunit 1 (*COI*) gene and internal transcribed spacer (ITS) rDNA regions supported the fact that *Eustrongylides* species collected from various fishes were divided into 3 clades. On the basis of ITS divergence it was concluded that clade 1 constitutes cryptic species while clades 2 and 3 might indicate the same species. Mazzone et al. (15) showed that their sequences was grouped in the clade 3 of Xiong et al. (22) was identified as *E. excisus* which was validated by morphological data also. Thus phylogenetic evidence helps in proper identification of larval specimens in addition to morphological data.

CONCLUSION

The present study characterizes *Eustrongylides* sp. by morphological and molecular techniques on the fish *C. punctata* from West Bengal in details which were not elaborated earlier. Further studies are required for understanding the phylogenetic

Table 1. Distance matrix based on nucleotide sequence homology (using Kimura 2 parameters) formed using ten sequences along with sequence KJ458967

	1	2	3	4	5	6	7	8	9	10
KJ458967		0	0.001	0.001	0.002	0.001	0	0.004	0.005	0.005
GQ215514	0		0.001	0.001	0.002	0.001	0	0.004	0.005	0.005
GQ215572	0.001	0.001		0.002	0.002	0.002	0.001	0.005	0.005	0.005
GQ215552	0.001	0.001	0.002		0.002	0.002	0.001	0.005	0.005	0.005
GQ215559	0.002	0.002	0.003	0.003		0.002	0.002	0.005	0.005	0.005
GQ215540	0.002	0.002	0.003	0.003	0.005		0.001	0.005	0.005	0.005
GQ215533	0	0	0.001	0.001	0.002	0.002		0.004	0.005	0.005
GQ215502	0.021	0.021	0.022	0.022	0.023	0.023	0.021		0.007	0.007
GQ215551	0.021	0.021	0.022	0.022	0.023	0.023	0.021	0.036		0.001
GQ215577	0.022	0.022	0.023	0.023	0.024	0.024	0.022	0.037	0.001	
GQ215528	0.024	0.024	0.025	0.025	0.027	0.027	0.024	0.04	0.006	0.007

Table 2. Comparative chart of morphometric charecters of larval stage of *Eustrongylides* sp. in fishes

Morphometric charecters	*Eustrongylides* sp. Moravec et al. (11), (mm)	*Eustrongylides* excises Čabrilo et al. (18), (mm)	*Eustrongylides* sp. Present study (mm)
Body length	32-50	27-40	17-20 (0.1375±0.89)
Body width	0.381-0.639	0.2 to 0.35	0.19-0.23 (1.177±0.072)
Buccal cavity	0.095-0.177	0.099	0.060-0.070 (3.467±0.972)
Length of oesophagus	8.704-11.628	2.9-5.0	4.25-6.0 (2.177±1.94)
Nerve ring	0.163-0.258	-	0.086-0.101(3.027±1.52)

position and enriching the fish database for parasites affecting common edible fishes like *C. punctata*.

*ACKNOWLEDGMENT

Authors are thankful to the late Prof P.K Bandyopadhyay, Department of Zoology, University of Kalyani, Kalyani for providing the all laboratory facilities during the research period. The authors would also like to thank the Director, Centre for Research of Nanoscience and Nanotechnology (CRNN), University of Calcutta for providing technical support for carrying out the scanning electron microscopy study.

*Ethics

Ethics Committee Approval: As per CPCSEA instruction's protocol for experimentation on fishes, does not require approval from ethical committee.

Informed Consent: There are no human patients in the study.

Peer-review: Internally peer-reviewed.

*Authorship Contributions

Concept: I.K., Design: D.R.M., I.K., Data Collection or Processing: I.K., Analysis or Interpretation: I.K., Literature Search: D.R.M., Writing: I.K.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

REFERENCES

1. Paperna l. Parasites, infections and diseases of fishes in Africa: An update. RFAO/CIFA Technical Paper No 31; 1996.
2. Roberts lS, Janovy J. Gerald D Schmidt and Larry S. Roberts' Foundation of Parasitology. McGraw-Hill International Editions. 6th Edition. Boston; 2000.
3. Dawes B. The Trematoda with special reference to British and other European forms. Cambridge University Press, Cambridge: England; 1946. p644.
4. Hoffman GL, Bauer ON. Fish parasitology in water reservoirs: A review. In G.E. Hall (ed) Reservoir fisheries and limnology. American Fisheries Society 1971; 56: 78-89.
5. Karve JN. Some parasitic nematodes of frogs and toads. Ann Trop Med PH 1930; 24: 481-91.
6. Agarwal V. Studies on some nematode parasites of freshwater fishes from Lucknow. Ann Parasitol Hum Comp 1966; 41: 217-37.
7. Soota TD. Studies on nematode parasites of Indian vertebrates I. Fishes Rec Zool Surv India Occ Paper 1983; 352.
8. Yamaguti S. The Nematodes of vertebrates. In: Systema Helminthum. Volume III. John Wiley and Sons: New York; 1961.
9. Moravec F. Parasitic nematodes of freshwater fishes of Europe. Kluwer Academic Publishers, Dordrecht/Boston: London; 1994.
10. Anderson RC. Nematode parasites of vertebrates their development and transmission. 2nd Edition. CABI Publising; 2000.
11. Moravec F, Nie P, Wang G. Some nematodes of fishes from central China, with the redescription of Procamallanus (Spirocamallanus) fulvidraconis (Camallanidae). Folia Parasitol (Praha) 2003; 50: 220-30.
12. Xiong F, Wang GT, Wu S, Nie P. Development of Eustrongylides ignotus (Nematoda: Dioctophmida) in domestic ducks (Anas Platyrhynchos domestica L. J Parasitol 2009; 95: 1035-9.
13. Novakov N, Bjelic-Cabrilo O, Cirkovic M, Jubojevic D, Lujic J, Davidov I, et als. Eustrongylidosis of European catfish (Siluris glanis). Bulgarian Journal of Agricultural Science 2013; 19: 72-6.
14. Llchtenfels JR, Pilitt PA. Eustrongylides sp. (Nematoda: Dioctophymatoidea): Differentiation of third and fourth-Stage larvae from killifish, Fundulm sp, collected in Chesapeake Bay Area, U.S.A. Proc Helminthol Soc Wash 1986; 53: 144-8.
15. Mazzone A, Caffara M, Gustinelli A, Agnetti F, Sgariglia E, Lo Vaglio G, et al. Morphological and molecular characterization of larval and adult stages of Eustrongylides excisus (Nematoda: Dioctophymatoidea) with histopathological observations. J Parasitol 2019; 105: 882-9.
16. Gupta N. Light and scanning electron microscopic studies on eustrongylides exciscus larvae (Nematoda: Dioctophmida) from Channa punctatus Bloch from India. Pak J Zool 2019; 51: 159-66.
17. Crites. Clear Technical Report No. 258, Ohio State University, Center for Lake Erie Area Research, Columbus: Ohio; 1982.p.83.
18. Čabrilo OB, Novakov N, Ćirković M, Kostić D, Popović E, Aleksić N, et al. The first determination of Eustrongylides excisus Jägerskiöld, 1909- larvae (Nematoda: Dioctophymatidae) in the pike-perch Sander lucioperca in Vojvodina (Serbia). Helminthologia 2013; 50: 291-4.
19. Measures LN. Revision of the genus Eustrongylides Jägerskiöld, 1909 (Nematoda: Dioctophymatoidea) of piscivorous birds. Can J Zool 1988; 66: 885-95.
20. Measures LN. The development of Eustrongylides tubifex (Nematoda: Dioctophymatoidea) in oligochaetes. J Parasitol 1988b; 74: 294-304.
21. McManus DP, Bowles J. Molecular genetic approaches to parasite identification: their value in diagnostic parasitology and systematics. Int J Parasitol 1996; 26: 687-704.
22. Xiong F, Li WX, Wu SG, Zou H, Wang GT. Molecular phylogeny and host specificity of the larval Eustrongylides (Nematoda: Diotophmidae) from freshwater fish in China. J Parasitol 2013; 99: 137-44.