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Morphological and Molecular Characterization of *Euclinostomum heterostomum* (Digenea: Clinostomatidea) Metacercariae in *Channa punctata* from Uttar Pradesh, India

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Abstract: Metacercariae of *Euclinostomum* species have been reported encysted in the kidneys, liver, and muscles of various fish hosts, whereas the adult worms are typically found in the mouth, pharynx, larynx, trachea, and upper esophagus of piscivorous birds. In this study, metacercariae identified as *Euclinostomum* sp., based on both morphological characteristics and molecular data, were collected from liver of *Channa punctata* in the Meerut region (India). In phylogenetic analysis of partial ITS-5.8S-ITS region of rDNA, the isolates of *Euclinostomum* obtained in this study formed a single, monophyletic group with other *Euclinostomum* sp. from Thailand, Italy and India, respectively. Morphology combined with molecular analysis confirms that the recovered parasites belong to the species *Euclinostomum heterostomum.*

Keywords: *Euclinostomum*, *Channa punctata*, Molecular, Trematode, Morphology, Phylogeny

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Introduction

Trematodes belonging to the genus *Euclinostomum* Travassos (1928) occur as adults in birds and as larvae (metacercariae) encysted in various organs and visceral mesenteries of freshwater fishes (Dubois, 1930; Dollfus, 1932; Tubangui and Masilungan, 1935; Bhalerao, 1942; Bilqees, 1972; Yamaguti, 1972; Bilqees *et al.*, 2011). Subfamily Euclinostominae was erected by Yamaguti, 1958 to accommodate *Euclinostomum* Travassos (1928) as the type genus and *Euclinostomum heterostomum* the type species. Members of thegenus have peculiar features such as lateral branches of caeca. This species was initially described by Rudolphi (1809) as *Distoma heterostomum*, found in the esophagus of *Ardea purpurea* L. Later, Braun (1899) synonymized

Distoma with *Clinostomum*, and subsequently, Travassos (1928) established the genus *Euclinostomum* to include *E. heterostomum.* Various species within the genus *Euclinostomum* have been identified based on their metacercarial stage, which forms cysts in the kidneys, liver, and muscle tissues of fish. The taxonomic history of *Euclinostomum* species is marked by the establishment of species based on characteristics that some researchers regard as uncertain or unreliable, leading to the possibility that several of these species may be classified as species inquirendae.

All confirmed records of *Euclinostomum* originate from the Old World (Table 1). Reports of its presence in Old World fish species introduced to South America, as cited by Pinto *et al.*(2014) are considered to be an"error in writing" (H. A. Pinto, pers. comm.). Fischthal and Kuntz (1963) were the first to describe the metacercarial stage of *Euclinostomum heterostomum* from *Tilapia* species and *Clarias* sp. collected in Egypt. In addition, these authors reviewed the *Euclinostomum* species described up to that time, synonymizing *Euclinostomum indicum* Bhalerao (1942) with *E. heterostomum*, and *Euclinostomum channai* Jaiswal (1957) with *Euclinostomum heptacaecum* Jaiswal (1957). Two new species, *viz; Euclinostomum dollfusi*, originally described by Dollfus (1950) as *E. heterostomum*, and *Euclinostomum vanderkuypi*, initially described by Van der Kuyp (1953) under the same name, were also proposed by Fischthal and Kuntz (1963). Ukoli (1966) reported the presence of *Euclinostomum* metacercariae in *Tilapia* species collected from Ghana. He described only two valid species *Euclinostomum multicaecum* (Tubangui & Masilungan, 1935) and the type species *E. heterostomum* and synonymized all other species described up to that point with these (Ukoli, 1966).

Bilqees (1972) introduced three new species of *Euclinostomum* such as *E. gastrocaecum*, distinguished by a prominent "stomach portion" surrounded by numerous unicellular glands and 7–9 unequal lateral diverticula; *E. heterocaecum*, characterized by short diverticula in the pre-acetabular region and an ovary positioned slightly to the left of the midline; and *E. nephrostomum*, noted for its bilobed acetabulum and differing lateral cecal branches before and after the testes. Additionally, a subspecies, *Euclinostomum indicum marulii*, was described based on features such as 9–10 lateral cecal branches, a larger body size, and a distinct sucker size ratio compared to *E. indicum*. Interestingly, distinguishing features of these species all fall within the range of morphological variation previously reported by Ukoli (1966) for *E. heterostomum*. Tendeiro *et al.* (1974) described *Euclinostomum lauroi* from *Pelecanus onocrotalus* L. in Mozambique, distinguishing it from the type species by its larger body size (9.86–18.21 mm) and the positioning of the gonads in the posterior half of the body. Despite ongoing debate regarding the reliability of pharynx presence as a taxonomic feature (Dönges, 1974), Bhutta and Khan (1975) described *Euclinostomum minutus* from *Ardea cinerea* L. in Pakistan, primarily based on the absence of a pharynx a structure present in *E. heterostomum* and other related species. *Euclinostomum ardeolae* was established by El-Naffar and Khalifa (1981) based on fine tegumental spines in the adult stage, slight sacculation in the pre-acetabular portions of the ceca, and comparatively larger gonads, which makes it different from the type species. Using metacercariae obtained from *Channa gachua* Hamilton, 1822, collected in the same region of Pakistan, Bilqees *et al.* (2011) described *Euclinostomum sindhensis*, while Shaikh *et al.* (2011) introduced *Euclinostomum robustum*. Later authors mentioned that the primary distinction between the two species lies in the absence of a pharynx in *E. robustum* and the absence of an esophagus in *E. sindhensis*. Furthermore, the presence of a prepharynx in both species distinguishes them from *E. heterostomum*.

Integrating molecular and morphological methods provides an effective means of clarifying taxonomic ambiguities among these species and those classified as species inquirendae. Ideally, genetically distinct species are identified and

Table 1: Hosts and geographical distribution of *Euclinostomum* species

	***Euclinostomum* spp.**	**Host**	**Locality**	**Reference**
1.	***E. heterostomum*** Rudolphi, 1809	*Ardea purpurea, A. cinerea, Nyctocorax griseus*	Italy	Stossich (1898); Braun (1901)
		A. cinerea	Czech Republic	Macko (1960, 1961); Sitko (1999)
		A. cinerea	Poland	Sulgostowska and Czaplinska (1987)
		A. cinerea	European USSR	Ivanov *et al.* (2002)
		A. purpurea	Bulgaria	Chipev and Kostadinova (1996)
		A. purpurea	Hungary	Murai *et al.* (1983)
		A. cinerea	Slovak Republic	Macko (1960, 1961, 1962)
		A. cinerea	Spain	Nogueserola *et al.* (2002)
		Clarias gariepinus, *Oreochromis mossambicus*	South Africa	Khalil (1971); Britz *et al.* (1985)
		C. gariepinus	South Africa	Mashego and Saayman (1989)
		Ardeola ralloides	Niger	Dollfus (1932)
		A. goliath	Belgian Congo	Dollfus (1933)
		Anabas testudineus, *A. cinerea*, *Tilapia nilotica*, *T. zillii*, *Clarias sp.*, *Bubulcus ibis*	Sulawesi	Van der Kuyp (1953)
		Tilapia nilotica, *T. zillii*, *Clarias sp.*, *Bubulcus ibis*	Egypt	Fischthal and Kuntz, (1963); Khalil, (1971); Taher (2009)
		T. zillii, *T. heudeloti*, *Anhinga rufa rufa*, *Phalacrocorax africanus africanus*	Ghana	Ukoli, 1966
		T. zillii	Nigeria	Dönges (1974); Echi *et al.* (2012)
		Bubulcus ibis	Liberia	Dennis and Sharp (1973)
		O. mossambicus	Malawi	Paperna (1980)
		O. mossambicus, *A. cinerea*	Mozambique	Britz *et al.* (1984)
		O. mossambicus, *A. cinerea*	Mozambique	Britz *et al.* (1984)
		O. niloticus	Ethiopia	Eshetu and Mulualem (2003)
		T. nilotica, *T. zillii*, *Barbus canis*, *O. aureus*, *Sarotherodon galilaeus*	Israel	Paperna (1964); Finkelman (1988); Dzikowski *et al.* (2003)
		Anas platyrhynchos domesticus, *Ardeola grayi*, *B. ibis*, *Columba livia domestica*, *Gallus domesticus*, *Pelecanus philippensis*, *Channa orientalis*, *C. punctatus*, *C. striata*	India	Odening (1962); Sinha *et al.* (1988); Jhansilakshmibai and Madhavi (1997);

				Shyamasundari and Rao (1998)
		C. punctatus	India	Vankara *et al.* (2011)
		C. marulius	Pakistan	Jafri (1993)
		C. punctatus, *C. striata*	Bangladesh	Chandra and Banerjee (1993); Arthur and Ahmed (2002)
		Apistogramma ramirezi	Hong Kong	Kazacos and Appel (1983)
		Trichopsis vittata, *T. scalleri*, *Betta splendens*, *B. imbellis*, *Poecilia reticulata*	Thailand	Purivirojkul and Sumontha (2013); Suanyuk *et al.* (2013)
		Clarias batrachus	Philippines	Cauyan *et al.* (2013)
2.	***E. multicaecum*** Tubangui and Masilungan, 1935	*Ophiocephalus striatus*	Philippines	Tubangui and Masiluñgan (1935); Velasquez (1959)
		A. purpurea	Indonesia	Purnomo *et al.* (1978)
		Channa punctata, *C. striata*, *Heteropneustes fossilis*, *Mystus tengara*, *Nandus nandus*, *Ompok pabda*	Bangladesh	Chandra (1983, 1984); Huq *et al.* (1983)
		C. striatus	India	Ramasamy *et al.* (1998)
3.	***E. gastrocaecum*** Bilqees, 1972	*C. marulius*	Pakistan	Bilqees (1972); Jafri (1993)
4.	***E. heterocaecum*** Bilqees, 1972	*C. marulius*, *C. punctatus*	Pakistan, India	Bilqees (1972); Jafri (1993)
5.	***E. nephrostomum*** Bilqees, 1972	*Ophiocephalus marulius*	Pakistan	Bilqees (1972)
6.	***E. indicum marulii*** Bilqees, 1972	*O. marulius*, *O. striatus*, *O. gachua*	Pakistan	Bilqees (1972)
7.	***E. lauroi*** Tendeiro, Travassos and Fazendeiro, 1974	*Pelecanus onocrotalus*	Mozambique	Tendeiro *et al.* (1974)
8.	***E. minutus***Bhutta and Khan, 1975	*A. cinerea*	Pakistan	Zaidi and Khan (1975)
9.	***E. ardeolae*** El-Naffar and Khalifa, 1981	*T. nilotica*, *Ardeola ibis ibis*	Egypt	Soliman and El-Damarany (1995); Shaheen *et al.* (2006); Taher (2009)
10.	***E. sindhensis*** Bilqees *et al.*, 2011	*C. gachua*	Pakistan	Bilqees *et al.* (2011)
11.	***E. robustum*** Shaikh *et al.*, 2011	*C. gachua*	Pakistan	Shaikh *et al.* (2011)

matched with existing names or formally described species based on their morphological characters. In the present study, ITS1-5.8S-ITS2 region of rDNA were amplified for the metacercariae of *Euclinostomum heterostomum.* The result of molecular analysis supported the morphological study.

Materials and Methods

Parasite Collection and Morphological study

In the present study, host specimens (*Channa punctata*) were purchased from local fishermen of Meerut (290 23N, 790 11'E), Uttar Pradesh, India, during June 2022 to July, 2023. Fish were dissected, and their internal organs were investigated under dissecting microscope to detect any parasitic infection. During investigation, liver was found infected with cysts of metacercariae, which were removed carefully using needle and placed in a physiological saline solution 0.9% for some time. Live as well as fixed specimens were used for morphological studies. Some parasites were fixed in 70% ethanol for permanent slide preparation and some of them were fixed in 95% ethanol for molecular study. For permanent slide preparation, ethanol fixed specimens were stained with acetocarmine, dehydrated with ascending grades of ethanol, cleared in xylene and mounted in Canada balsam. Permanent slides were deposited in the museum, Department of Zoology, Chaudhary Charan Singh University, Meerut (U.P.), India. Photographs were taken with the help of Nikon Eclipse Ts2 binocular microscope. Measurements were taken from digital images using NIS Elements 5.10 image analysis software. All measurements were taken in micrometer unless otherwise stated.

DNA Isolation and Amplification

For genomic DNA extraction, specimens fixed in 95% ethanol were used. Isolation of DNA was done using DNeasy™ blood and Tissue Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. ITS1-5.8S-ITS 2 gene cluster of ribosomal DNA region were amplified. ITS1-5.8S-ITS2 gene were amplified using primer set D1(F) 5′-AGGAATTCC TGGTAAGTGCAAG-3′ (forward) and D2(R) 5′-CGTTAC TGAGGGAA TCCTGG-3′ (reverse) primers as suggested by Hillis and Dixon (1991). Purification of amplified PCR products was done using Purelink™ Quick Gel Extraction and PCR Purification Combo Kit (Invitrogen, Löhne, Germany) following manufacturer's instructions. Using same sets of primers, sequencing reaction were performed in both directions using ABI Big Dye Terminator Cycle Sequencings Reaction Kit (Applied Biosystems, Foster City, California, USA), using D1(F) and D2(R) for ITS1-5.8S-ITS2 clusters to generate long stretches of sequences.

Phylogenetic Analysis

Using BioEdit software, the resulting sequences were visualized, assembled, and modified. In order to retrieve highly similar sequences of *Euclinostomum* sp., BLASTn searches were performed at GenBank for newly generated sequences. The sequences were then deposited in the GenBank. Alignments of sequences were done separately using cluster W (Thompson *et al.*, 1994) implemented in the MEGA 6.06 (Tamura *et al.*, 2013). Phylogenetic analysis was done by MEGA 6.06 using maximum likelihood (ML) and neighbour joining (NJ) tree method. Both methods yielded the same result therefore, only Maximum Likelihood method is presented here. Invariant sites and gamma-distributed rate among site variation (GTR+I+G) were chosen as the best nucleotide substitution models for ITS cluster (ITS1-5.8S-ITS2) data sets. The nodal support values were tested using 500 bootstrap pseudo-replicates and pairwise distances of each region were estimated. *Tylodelphys clavata* (MW135176) and *Diplostomum baeri* (AY123042) were selected as out groups for the ITS1-5.8S-ITS2 region.

Results

In the present study, host *Channa punctatus* was found infected with metacercariae belonged to the genus *Euclinostomum* according to their general clinostomid morphology and presence of bifurcating caeca. The species was initially recognized as *E. heterostomum* due to its morphological similarities to metacercariae described by Jhansilakshmibai and Madhavi (1997).

Morphological analysis

Description is based on measurements of five stained specimens (Fig. 1). Metacercariae were found encysted in the liver of the host *Chaana punctata*. Width of oral collar, measuring 526.51

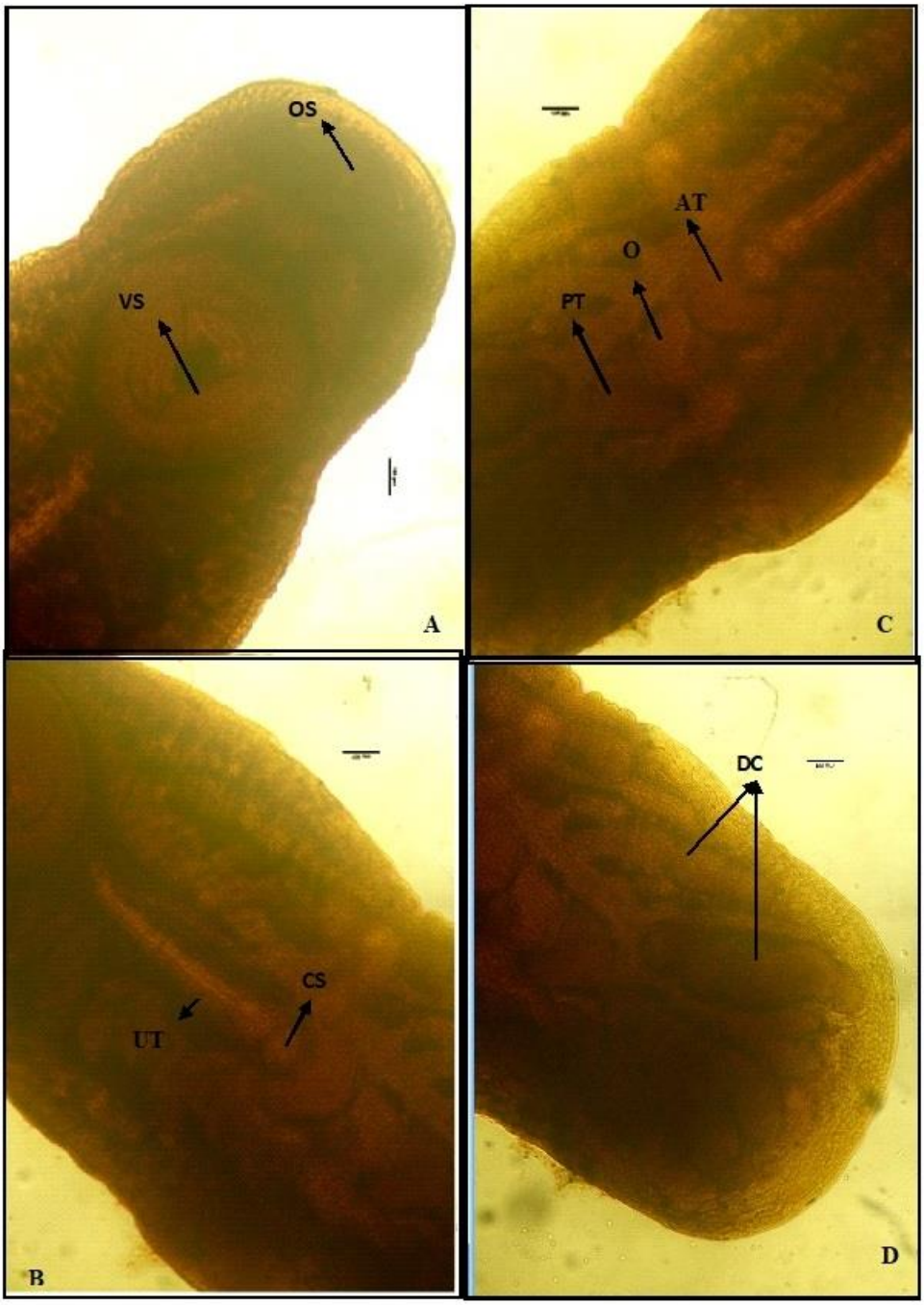

Fig. 1: Metacarcaria of *Euclinostomum heterostomum* from liver of *Channa punctata*. (A) Anterior end showing oral sucker; (B) showing uterus and cirrus sac; (C) showing anterior testis, ovary and posterior testis; and (D) Posterior end showing diverticulated caeca and excretory pore. Scale bar A, B, C, and D=100.

(357.5-684.14). The body stout and fleshy, tongue-shaped (linguiform), tapering toward the anterior end and slightly rounded at the posterior, measuring 3117.45 (1493.95-4284.54) in length and1177.94 (530.5-1620.33) in width. The greatest width occurs at the pre-acetabular region and around the testicular area. Smooth and thin cuticle. The oral sucker small and sub-terminal, measuring 223.12 (100.36-347.23) in length and 334.73 (199.27-407.61) in width. The strongly

muscular and well-developed ventral sucker, nearly round on the outer margin and heart-shaped internally, located at the end of the first quarter of the body measuring, 626.11 (388.63-877.64) in length and 611.54 (380.28-841.62) in width, shares lateral borders with the caeca. Distance between suckers 230.29(176.08-318.89). Pharynx not visible. The esophagus bifurcates into two long; diverticulated caeca that extend to the posterior end of the body. The caecal branches are elongated and lobed, resembling finger-like projections with slightly swollen tips. Asymmetrical testis, arranged in tandem, placed in the third quarter of body. Anterior testis wider than long, measuring, 219.38 (75.55-374.06) in length and 335.61 (149.46-555.5) in width. Posterior testis also asymmetrical, slightly lobed, measuring 212.55 (99.52-237.03) in length and 339.74 (125.04-522.55) in width. Inter testicular distance 258.81 (198.17-330.95). The ovary small, nearly spherical, and positioned laterally at the upper margin of the anterior testis, measuring 160.42 (39.64-251.51) in length and 251.51 (31.26-455.4) in width. Round cirrus sac, located in front of anterior testis, measuring 148.28(97.51-197.97) in length and 159.28 (102.92-240.02) in width. Ratio between cirrus pouch length and body length 0.049 (0.034-0.065). The uterus is long and intercecal, located in the center of the body, and extends anteriorly up to the ventral sucker, measuring 566.97 (468.5-695.33) in length and 75.14 (64.7-87.91) in width. Small and inconspicuous vitellaria. Excretory pore median, sub-terminal.

Taxonomic summary

Host: Striped snakehead *Channa punctata* (Bloch, 1793) (Perciformes - Channidae).

Locality: Meerut (290 23N, 790 11'E) Uttar Pradesh, India.

Site of infection and prevalence (P): Liver, 70 % (7 infected out of 10 hosts; 11 parasites were found from liver of 7 infected hosts).

Mean abundance (MA): 1.1.

Mean intensity (MI): 1.57.

Material deposited: The slides were deposited in the Museum, Department of Zoology, Chaudhary Charan Singh University Meerut Uttar Pradesh, India.

Representative sequences: The ribosomal gene cluster of ITS1-5.8S-ITS2 (845bp) of *E. heterostomum* was deposited in the GenBank under the accession number OR228470.

Phylogenetic Analysis

In the present study, partial ITS1- 5.8S-ITS2 sequences of rDNA from two isolates of *Euclinostomum* sp. were obtained and submitted to GenBank. The BLAST analysis revealed 99.64-99.52% maximum identity with those species of *Euclinostomum heterostomum* collected in India (MT785768) and Thailand (KC894799–KC894801). Phylogenetic analysis was done by using Maximum likelihood (ML) and Neighbour joining (NJ) analysis. *Tylodelphys clavata* (MW135176, MW001129) and *Diplostomum baeri* (AY123042) was chosen as out- group.

Phylogenetic tree analysis of ITS1- 5.8S-ITS2 region (Fig. 2) indicated that sequence generated during study were formed a monophyletic clade with other *Euclinostomum* sp. from Thailand, Italy and India. For comparison with other sequence of *Euclinostomum* there are two sequences available in NCBI from India (MT785768,KF781297) and other from Thailand, Italy, respectively. Further investigation with the use of pair-wise distance analysis revealed that ITS1- 5.8S-ITS2 sequence of *Euclinostomum heterostomum* TR-7 differ from other examined *Euclinostomum* sp. with intra-specific sequence divergence of 0.019 to 0.30 % from India, 0.019 to 0.022% from Thailand and 0.024 to 0.029 from Italy, respectively.

Discussion

Numerous freshwater fish species act as intermediate hosts for *Euclinostomum heterostomum*; however, both previous reports and current findings suggest that murrels (*Channa* spp.) are particularly susceptible and represent the most favorable second intermediate hosts in Indian freshwater ecosystems. In contrast, studies

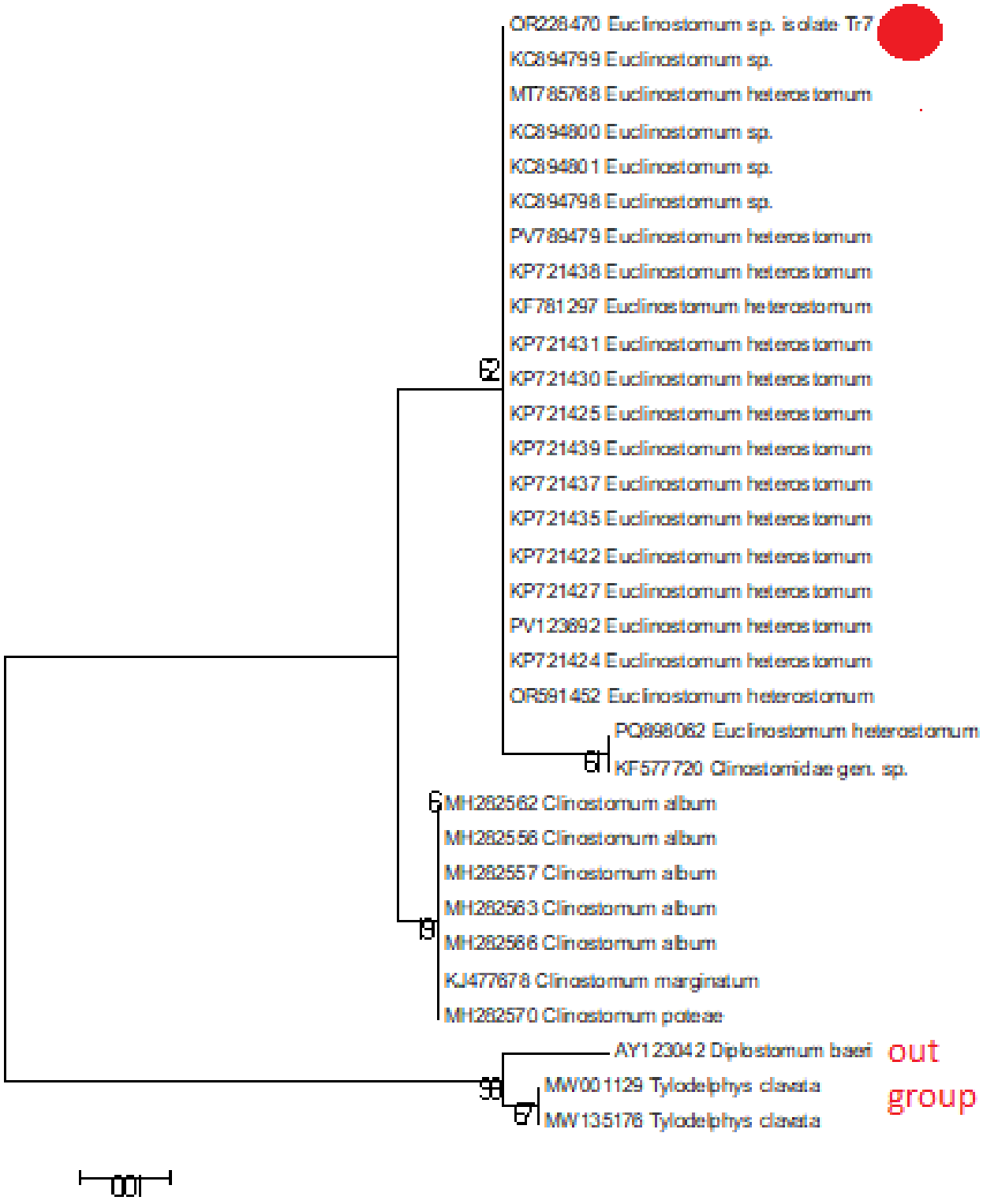

Fig. 2: Phylogenetic interrelationships inferred by Maximum Likelihood analysis for representatives of *Euclinostomum* species based on ITS1-5.8S-ITS2 sequences. The scale-bar indicates the number ofsubstitutions per site. Species names are provided after GenBank accession numbers.

from Egypt have identified *Tilapia* species as the primary second intermediate hosts for *E. heterostomum*. Interestingly, most fish species reported as second intermediate hosts possess accessory respiratory organs, though the significance of this association remains unclear. Another notable observation is the variation in tissue localization of metacercariae between the two *Channa* species (Jhansilakshmibai and Madhavi, 1997). In *C. punctatus*, metacercariae were found in the liver and kidney, whereas in *C. orientalis*, they were located within the muscle tissue. They causes pronounced histopathological damage, particularly in liver and kidney, which likely impairs overall fish health. The economic and food safety implications suggest that monitoring and control (at least via proper inspection and cooking) are important. Better taxonomic and ecological understanding would aid in managing its impacts. The factors influencing this differential site preference are yet to be elucidated.

Genus *Euclinostomum* was erected by Travassos (1928) under the subfamily Euclinostominae to include Clinostomid which have well developed diverticulated caeca. They occur as adults in birds and as larvae (metacercariae) encysted in various organs and visceral mesenteries of freshwater fish (Dubois, 1930; Dollfus, 1932; Tubangui and Masilungan, 1935; Bhalerao, 1942; Bilqees, 1972; Yamaguti, 1972; Bilqees *et al.*, 2011). Dubois (1930) reported *Euclinostomum clarias* from the body cavity of *Clarias angolense*; and also recorded *Euclinostomum heterostomum* from the pharynx of *Ardeola ralloides* of Niger Valley in 1932. The key of Clinostomidae was reviewed by Bear (1933) and he also developed a key for classification of *Euclinostomum* designating *Euclinostomum heterostomum* Rudolphi, 1809 as type species of this genus. Tubangi (1935) added *E. multicaecum* from muscular tissue of *Ophicephalus striatus* in Phillipine. Bhalerao (1942) described *E. indicum* metacercaria in the body cavity of *Ophicephalus punctatus*. Later, Jaiswal (1957) added three more forms from birds and fish of Hyderabad, India, *viz; E. bhagavantami, E. heptacaecum E. channai*. However, Yamaguti (1972) synonymized *E. bhagavantami* and *E. channai* with *E. heterostomum*. Along with these species, certain other species have been reported including *E. africanum* Stossich and Galli-Valerio (1906); *E. dollfusi* Fischthal and Kuntz (1963); *E. vanderkuypi* Fischthal and Kuntz (1963); *E. gastrocaecum* Bilqees (1972); *E. heterocaecum* Bilqees, (1972); *E. nephrostomum* Bilqees, (1972); *E. lauroi* (Tendeiro *et al*., 1974); *E. minutus* (Zaidi and Khan, 1975); *E. ardeolae* (El-Naffar and Khalifa, 1981) and *E. robustum* (Perveen, 2011). Many forms such as *E. dollfusi, E. vanderkuypi* and *E. bhagavantami* are considered as synonyms of *E. heterostomum.*

In the present study, taxonomic identities of the genus *Euclinostomum* reported from India were re-evaluated using an integrated approach that combined detailed morphological examination with molecular analyses. The morphological characteristics of *Euclinostomum heterostomum* TR-7 closely resembled the original description of *E. heterostomum* (Rudolphi, 1809) (Travassos, 1928; Jhansilakshmibai and Madhavi, 1997), in terms of the total body length, tandem arrangement of testes, bifurcating ceaca, and presence of numerous lateral branches from the caeca. Minor variations in body shape were noted; however, these differences are likely attributable to inconsistencies in fixation and mounting techniques employed during specimen preparation. A consistent observation in our measurements was that the specimens were smaller in size compared to those *Euclinostomum* metacercaria reported by Rai (1970) and almost similar with *E. heterostomum* described by Jhansilakshmibai and Madhavi (1997) in *Channa punctata* from muscle, coelomic cavity and liver, respectively.

Several species of the genus *Euclinostomum* have been described in India, identified either at the metacercarial or adult stage. These include *E. heterostomum* (Rudolphi, 1809), *E. indicum* (Bhalerao, 1942), *E. heptacaecum* (Jaiswal, 1957), *E. bhagavantami* (Jaiswal, 1957), and *E. channai*

(Jaiswal, 1957). In a comprehensive revision of the genus, Ukoli (1966) synonymized all Indian species of *Euclinostomum* with *E. heterostomum*, recognizing only two valid species: *E. heterostomum* and *E. multicaecum* (Tubangui and Masilungan, 1935). This taxonomic perspective is supported by subsequent studies, including those of Dennis and Sharp (1973), Caffera *et al.* (2016), Senapin *et al.* (2014) and is in agreement with the findings of the present investigation.Additionally, we consider the two species described by Bilqees (1972) from Pakistan *E. gastrocaecum* and *E. nephrostomum* to be synonyms of *E. heterostomum*. However, we regard *E. minutus* Zaidi & Khan, (1975) from Pakistan and *E. ardeolae* El-Naffar & Khalifa, (1981) from Egypt as valid and distinct species within the genus.

The morphological observations were further corroborated by molecular data. Over the past decade, the growing availability of genetic resources has significantly advanced our understanding of the diversity and phylogenetic relationships within clinostomid trematodes (Razo-Mendivil *et al.*, 2013; de León *et al.*, 2015a, b; Pinacho-Pinacho *et al.*, 2021).

Conclusion

The molecular and phylogenetic findings of the present study are consistent with those of Senapin *et al.* (2014), who examined the phylogeny of the genus *Euclinostomum* in *Trichopsis* and *Betta* fish. In the phylogenetic analysis of ITS1-5.8S –ITS2 sequence revealed a monophyletic clustering of *Euclinostomum* species with other *Euclinostomum* spp. from India (MT785768, KF781297) and Thailand (KC894799–KC894801) whereas *Euclinostomum* and *Clinostomum* formed distinct sister monophyletic clade. In the present study morphology combined with molecular analysis confirming that the genus *Euclinostomum* represented in the current samples corresponds to a single species, *E. heterostomum*.

Ethical Statement

The authors confirm that all ethical issues have been dealt with the research ethics guidelines

provided by the Department of Zoology, Chaudhary Charan Singh University, Meerut 250004, Uttar Praddesh, India.

Author Contributions

Each author has contributed equally in designing the research, conducting thorough analysis, drafting the manuscript, and refining its content. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

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