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Phylogenetic analysis of the *Dactylogyroides longicirrus* (Monogenea: Dactylogyridae) based on the 18S and ITS 1 ribosomal genes

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Abstract:
The present study describes the molecular phylogenetic analysis of *Dactylogyroides longicirrus* (Monogenea: Dactylogyridae) infecting the gill filaments of fish *Puntius sophore* from the site Guwahati, Assam, India. The parasite *Dactylogyroides longicirrus* (Tripathi, 1959) Gusev, 1976 from Northeast Indian region is presented based on sequence data of a 738 base-pair fragment of ribosomal 18S small subunit and first internal transcribed spacer (ITS 1). Phylogenetic relationships were inferred using neighbour joning and maximum parsimony methods and the results support the validation of *D. longicirrus*. The study is also supported by secondary structure model prediction by using minimum free energy which can be considered a promising tool for monogenean species identification. This is the first report of this parasite from Northeast region of India, with this, the 18S and ITS 1 rDNA region amplified in the study is also the first sequence of the genus *Dactylogyroides*.

Keywords: *Dactylogyroides*, Fish, Ribosomal 18S small subunit, Assam, India.

Background:
Platyhelminthes are among the most phylogenetically basal group of bilateral animals **[1, 2]**. Although, with the increased used of molecular methods there has been an explosive interest in the systematics of Platyhelminthes. Molecular systematic methods have also been used to assess relationships within Platyhelminthes **[3-11]**. 18S and ITS 1 ribosomal DNA sequences evidence was frequently used for assessing the phylogeny of monogenean parasites **[12-16]**. During our survey of freshwater fish fauna for monogenean infection, *Dactylogyroides longicirrus* **[17-18]** was found to be infected, gill filaments of host fish *Puntius sophore* from river Brahmaputra at the site Guwahati, Assam, India. Currently, due to the lack of parasite surveys only a little bit is known of the monogenean fauna from this region of India. Although, identification of *Dactylogyroides longicirrus* **[17-18]** was sufficient earlier using morphological features but the present study, based on a combination of molecular biology in addition to secondary structure prediction reported here to determining the phylogenetic analysis of this parasite.

Methodology:
Sampling site, Host and Parasites
Brahmaputra River enters India and further continues its journey to the Bay of Bengal through Bangladesh. During a survey, host species, *Puntius sophore* was caught from the river Brahmaputra at the site Guwahati (26°11'N and 91°44'E) **(Figure 1)**. Immediately after capture, the fish were killed by a sharp blow on the top of the head and dissected. Monogeneans were collected from the gills according to the method suggested by Malmberg **[19]**. This parasites were identified with the help of morphology of the haptoral hard parts and copulatory complex **(Figure 2).** Study of morphology of hard parts were analyzed as described by Malmberg **[19]**. The slides have been deposited in the museum of the Department of Zoology (voucher number HS/Monogenea/2012/12), Chaudhary Charan Singh University, Meerut (U.P.), India.

Figure 1: Location of river Brahmaputra in Assam, Guwahati and its course through India.

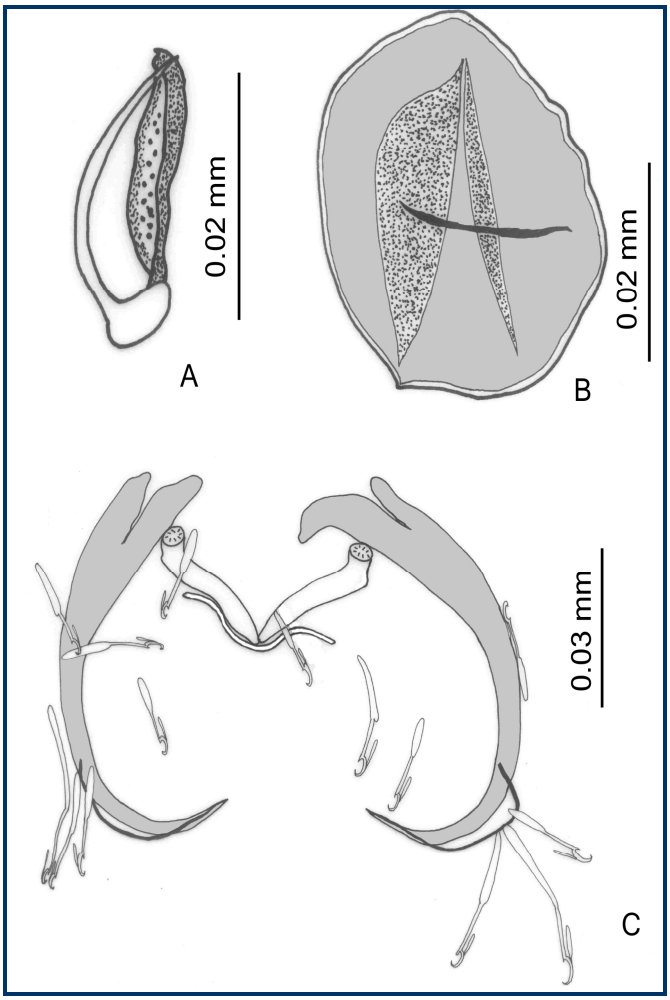

Figure 2: Morphology of *D. longicirrus* (Tripathi, 1959) Gusev, 1976 A. copulatory complex; B. Egg; C. Haptoral armature.

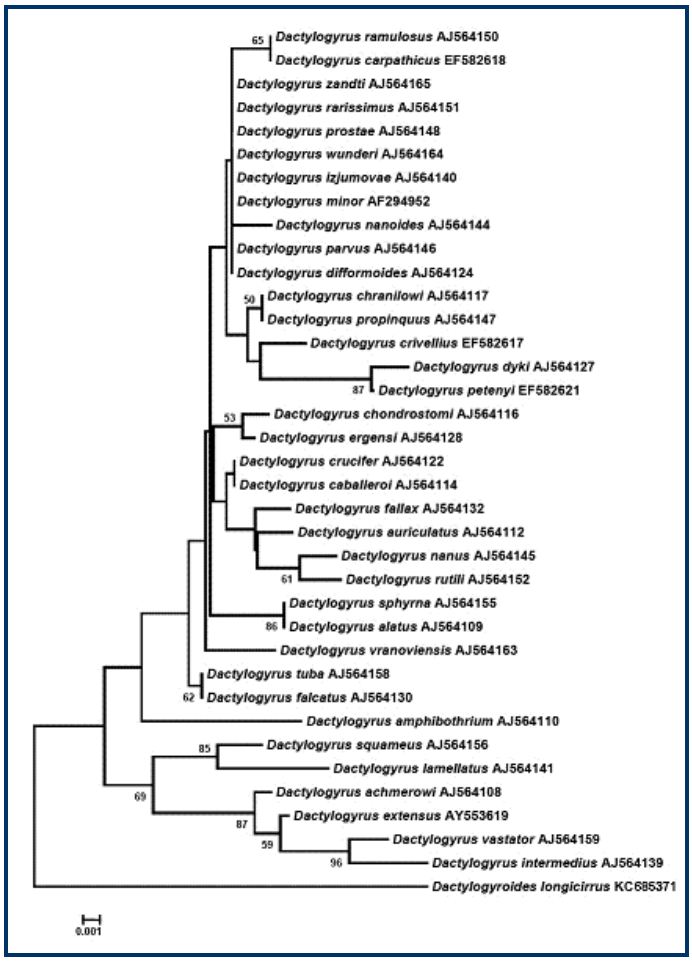

Figure 3: Phylogeny of 18S and ITS 1 rDNA of *D. longicirrus* (Tripathi, 1959) Gusev, 1976 obtained by Neighbour joining (NJ).

Amplification, sequencing and phylogenetic analysis

The ribosomal DNA of parasite was extracted using DNeasy Tissue Kit (Qiagen). The purified DNA obtained was suspended in buffer and stored -20^{0}C. The PCR amplification of 18S and ITS 1 ribosomal RNA gene was carried out by specifically designed primer, (forward, 5'-CGGTTGCAATTTTTATGTGG-3') and (reverse, 5'-GAGTGATCCACCACTTGCAG-3'). Reaction was performed in final 25 µl volume containing 3 µl of lysate, 10 X polymerase chain reaction (PCR) buffer, 1 unit of Taq polymerase (Biotools, Madrid, Spain), 0.4mM dNTP and 10 pM of each primer pair. PCR products were examined on 1.5% agarose–TBE (Tris-borate-EDTA) gels, stained with ethidium bromide and visualized under ultraviolet light. Amplification products were purified by a Chromous PCR clean up kit (#PCR 10, Chromous Biotech, Bangalore, India). Gel-purified PCR products were sequenced using a Big Dye Terminator version 3.1 cycle sequencing kit in ABI 3130 genetic analyser (Applied Biosystems) with the same primers. The closely related homologous sequences were identified by comparing the 18S and ITS 1 rRNA gene sequence of *D. longicirrus* with the monogenean sequences available at NCBI. ClustalW2 **[20]** was used to align all sequences with default settings. Phylogenetic trees were reconstructed using MEGA version 5 **[21]**. Phylogenetic analysis was performed based on neighbour-joining (NJ) and maximum-parsimony (MP) methods. In

reconstructing the NJ tree, the Kimura two-parameter model [**22]** was used to estimate the distances.

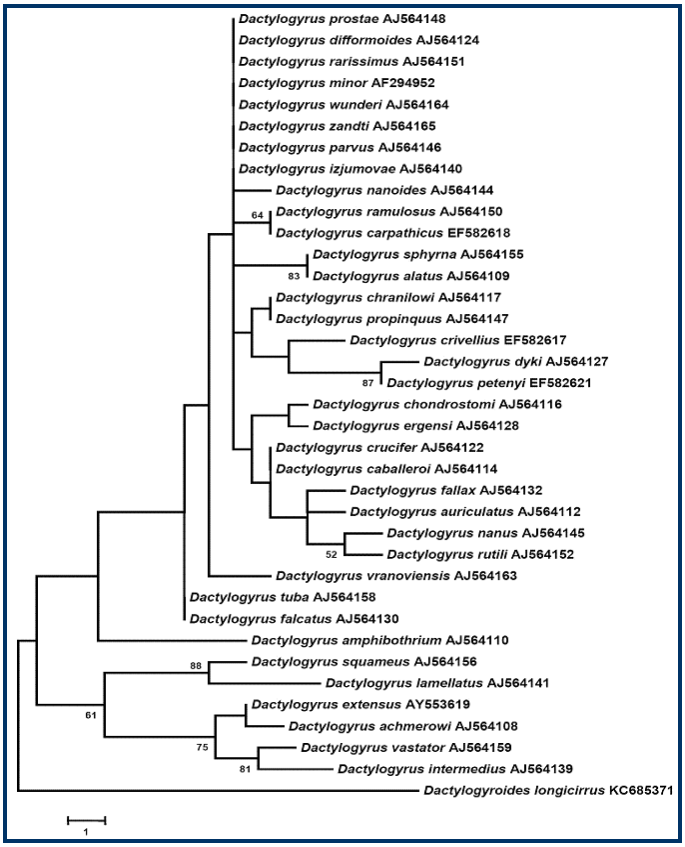

Figure 4: Phylogeny of 18S and ITS 1 rDNA of *D. longicirrus* (Tripathi, 1959) Gusev, 1976 obtained by maximum parsimony (MP). The scale bar indicates the proportion of the sites changing along the each branch.

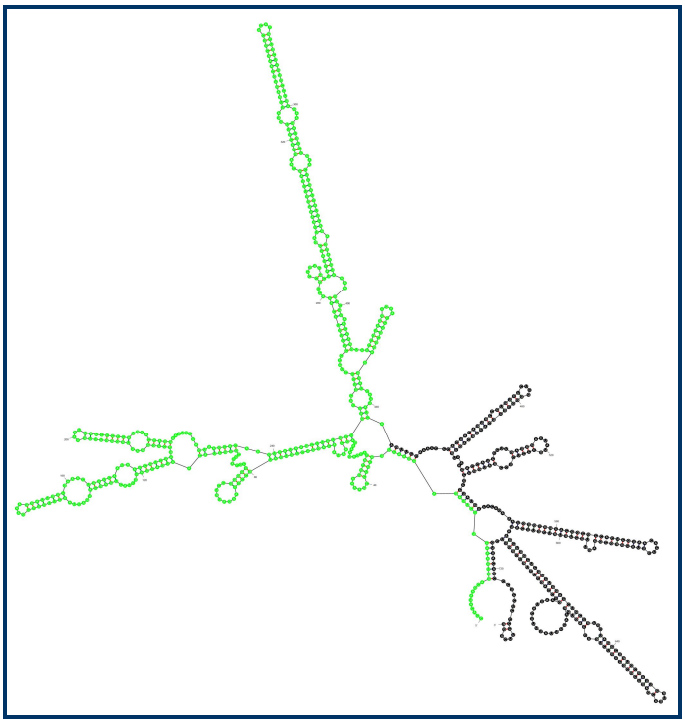

Figure 5: Schematic representation of 18S and ITS 1 rRNA predicted secondary structure for *D. longicirrus* (Tripathi, 1959) Gusev, 1976 reported from India. The base pairs in green showed arrangement of 18S sequence whereas base pairs in black showed ITS 1 region.

Prediction of secondary structure and analysis

An RNA secondary structure was predicted by using Mfold **[23]**. The inferred structure was subsequently examined for stems, loops and bulges. GC content is known to influence structural energy, since GC percentage was determined using a GC calculator. Energy levels of presumptive secondary structures were then calculated with Mfold **[24, 25]**.

Nucleotide accession number

The 18S and ITS 1 rRNA sequence of the parasite was deposited as GenBank ID: KC685371.

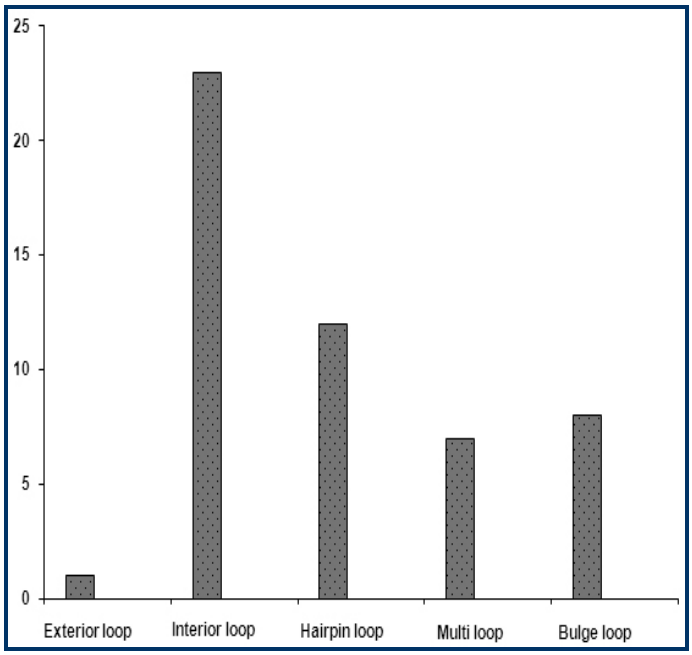

Figure 6: Distribution of different type of loops in the 18S and ITS 1 region of *D. longicirrus* (Tripathi, 1959) Gusev, 1976.

Results:

Molecular characterization

The 18S and ITS 1 rDNA sequence of *D. longicirrus* in BLAST analysis showed 97% maximum similarity with the sequences of species of genus *Dactylogyrus* available at NCBI. Phylogenetic relationship of the species *D. longicirrus* and related taxa are given in figure **(Figure 3 & 4)**. Phylogenetic analysis using the various methods like neighbour-joining (NJ) and maximum-parsimony (MP) showed that the topology is similar among the trees obtained **(Figure 3 & 4)**. The analysis revealed a close relationship of *D. longicirrus* with isolates of genus *Dactylogyrus* because the genus *Dactylogyroides* was originally differentiated by Gussev **[26]** from *Dactylogyrus* including the species i.e., *Dactylogyroides longicirrus* from India. Although, 28S rDNA partial sequence of the *D. longicirrus* was submitted to the GenBank under the accession no. GU903482. However, this analysis showed the first 18S and ITS 1 sequence for any species of the genus *Dactylogyroides* but to further strengthen the validation of species of genus *Dactylogyroides* more data is required from different species under this genus for comparative analysis.

Secondary structure analysis

Secondary structure was reconstructed from the 18S and ITS 1 sequence with highest negative free energy ΔG= -290.40 Kcal/mol of *D. longicirrus* to provide the basic information for phylogenetic analysis **(Figure 5)**. The secondary structural features of 18S and ITS 1 region as shown in the figure were analyzed based on conserved stems and loops. In the structure of *D. longicirrus* the orders of preference of loops in their number were interior loop, hairpin loop, bulge loop, multi loop and exterior loop **(Figure 6)**. The stems stabilize RNA secondary structure and the different features of the structure are: G+C content (%) = 51.8; number of GC = 382; AU = 356 and GU = 381. The Energy dot plot **(Figure 7)** represents the superposition of all possible folding and the different colors are used to indicate varying levels of sub optimality. The ss-count **(Figure 8)** showed the propensity of a base to be single stranded, and in a group of predicted folding measured by the number of times it is single stranded. The topology based only on the predicted RNA secondary structure of the 18S and ITS 1 region would help in future studies to resolve most relationships among the different species of this genus studied.

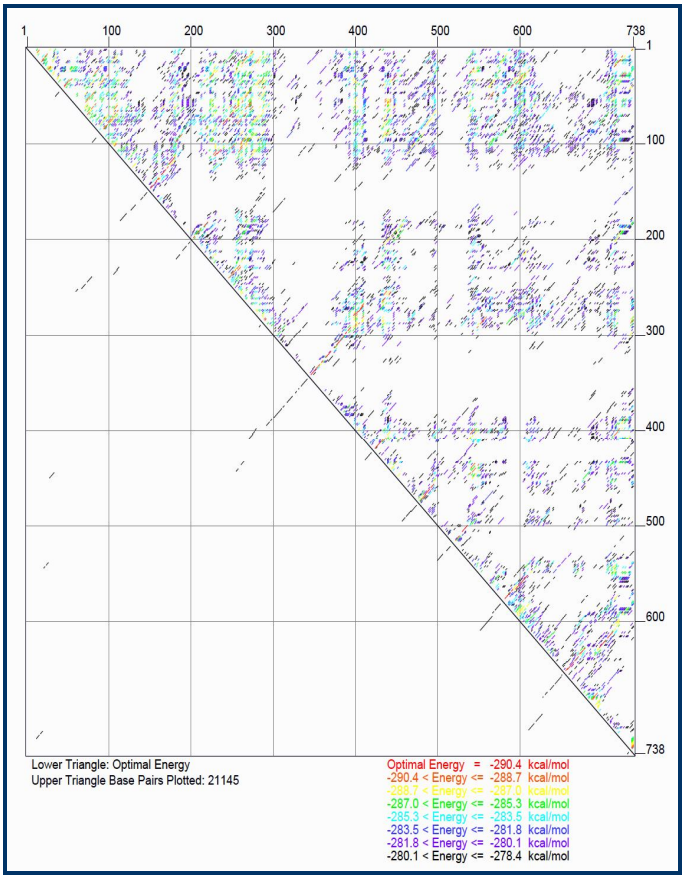

Figure 7: The corresponding energy dot plot for the annotated structure plot showed in the figure for *D. longicirrus* (Tripathi, 1959) Gusev, 1976.

Discussion:

Traditionally, monogenean classification was based, to a large extent, on the morphology of the sclerotized components of the haptoral parts. PCR technology and DNA sequencing techniques permit the identification of species more easily. The analysis of 18S and ITS 1 rRNA gene sequence has also revealed that the *D. longicirrus* showed 97% similarity with the closely related species of the genus *Dactylogyrus*. The present study, inferred from 18S and ITS 1 rDNA data depicted that *Dactylogyroides* and *Dactylogyrus* as genetically closely related sister taxa. Therefore, based on the molecular results, the investigator proposed that, the species *D. longicirrus* was correctly transferred in the genus *Dactylogyroides* by Gusev **[18]**. This study further indicated that molecular markers such as rDNA is useful for distinguishing sister genera or species. It may be helpful in discriminating species especially when morphological differences are often difficult to determine. Investigators also believes that such taxonomic revisions based on molecular biology will continue with the increasing number of species being used for molecular phylogenetic investigations in the future. To the best of my knowledge, there has been no such previous 18S and ITS 1 sequence of this species and even this genus on GenBank database.

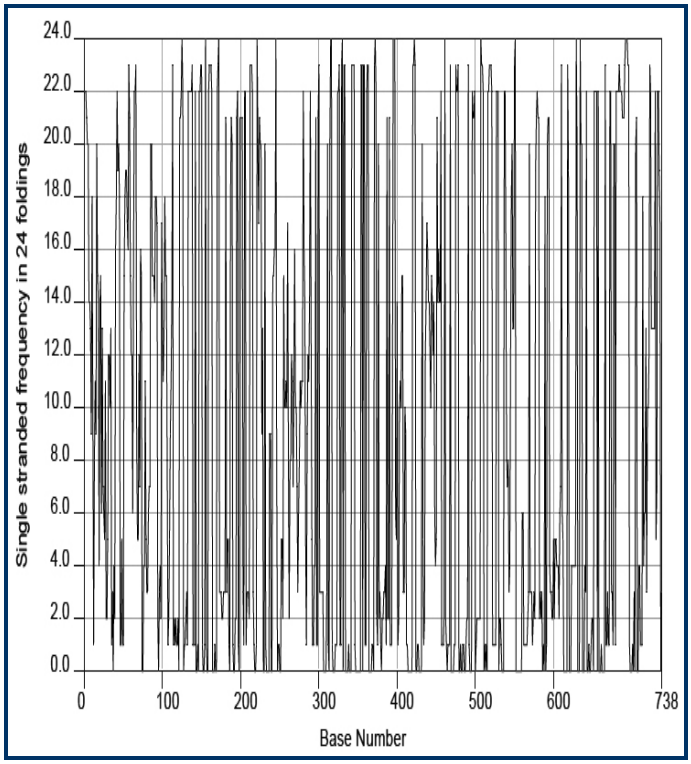

Figure 8: The figure showed the ss-count, it is included to assume the expected probability for each base of being single-stranded.

In phylogenetic studies, the molecules measurable structural parameters are used directly as specific characters to construct a phylogenetic tree. These structures are inferred from the sequence of the nucleotides, often using energy minimization **[27]**. In the study of phylogenetics, only the size variations of homologous structural segments are considered, whereas molecular morphometrics infers the folding pattern of RNA molecule. RNA secondary-structure models add significant dimensions to our understanding of the relationships among the sequence features and structural parameters that come into play in determining the structural energy. Therefore, structural model-based analyses of DNA sequence data have become increasingly important for phylogenetic inference. Incorporating secondary structure information will allow improved estimates of phylogeny among several *Dactylogyroides* species based on 18S and ITS 1 rDNA evidence

in future studies. Extension of these molecular data, in the number of species should allow molecular systematics to continue to make a significant contribution to elucidate the phylogeny of these fascinating organisms.

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References:

[1] Field KG *et al. Science*. 1988 **239**: 748 [PMID: 3277277]
[2] Morris SC, *Nature*. 1993 **361**: 219
[3] Qu LH *et al*. *Mol Biochem Parasitol.* 1986 **20**: 93 [PMID: 3736598]
[4] Ali PO *et al*. *Mol Biochem Parasitol*. 1991 **46**: 201 [PMID: 1922195]
[5] Baverstock PR *et al*. *Int J Parasitol*. 1991 **21**: 329 [PMID: 1894431]
[6] Korbsrisate S *et al*. *Gene*. 1991 **105**: 259 [PMID: 1937023]
[7] Riutort M *et al*. *Biochem Syst Ecol*. 1993 **21**: 71
[8] Blair D, *Int J Parasitol*. 1993 **23**: 169 [PMID: 8495999]
[9] Blair D & Barker SC, *Int J Parasitol*. 1993 **23**: 527 [PMID: 8354602]
[10] Johnston DA *et al*. *Parasitology*. 1993 **107**: 147 [PMID: 8414669]
[11] Rohde K *et al*. *Int J Parasitol*. 1993 **23**: 705 [PMID: 8300281]
[12] Cunningham CO *et al. Mol Biochem Parasitol.* 1995 **71**: 139 [PMID: 7630378]
[13] Desdevises Y *et al. Int J Parasitol.* 2000 **30**: 741 [PMID: 10856509]
[14] Poisot T *et al.* PLoS One. 2011 **6**: e26252 [PMID: 22022582]
[15] Li AX *et al*. *Mol Cell Probes*. 2005 **19**: 35 [PMID: 15652218]
[16] Paladini G *et al. Parasit Vectors*. 2011 **4**: 100 [PMID: 21658217]
[17] Tripathi YR, *Indian J Helminthol*. 1959 **9**: 1
[18] Gussev AV, *Indian J Helminthol*. 1976 **25**: 1
[19] Malmberg G, *Arkiv für Zoologi Serie*. 1970 **2**: 1
[20] Thompson JD *et al*. *Nucleic Acids Res*. 1994 **22**: 4673 [PMID: 7984417]
[21] Tamura K *et al*. *Mol Biol Evol*. 2011 **28**: 2731 [PMID 21546353]
[22] Kimura M, *J Mol Evol*. 1980 **16**: 111 [PMID: 7463489]
[23] Zuker M, *Nucleic Acids Res*. 2003 **31**: 3406 [PMID: 12824337]
[24] Jaeger JA *et al*. *Proc Nat Acad Sci U S A.* 1989 **86**: 7706 [PMID: 2479010]
[25] Zuker M *et al*. *Dordrecht The Netherlands, Kluwer Academic Publications* 1999 p. 11-43
[26] Gussev AV, *Fisheries Res Station Ceylon*. 1963 **16**: 53
[27] Zuker M, *Methods Mol Biol*. 1994 **25**: 267 [PMID: 7516239]

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