SUMMARY

The thesis entitled, “Monogenean fauna of Brahmaputra river system” dealing with the taxonomy, validation and phylogenetic position of dactylogyrids monogenean parasites harboring the gills of freshwater fishes of river Brahmaputra. Within this family, the parasites display the greatest diversity and complexity in the parasite community. The parasite communities on respective hosts display noteworthy richness. Species identification in this group of parasites is mainly based on the hard parts of attachment organs, where the shape of copulatory complex and haptoral parts have proven to be especially useful. It has been seen both experimentally and under natural condition that these hard parts show a high degree of variation in size and shape that is linked to other factors.

In this study, the investigator, evaluated the taxonomy of some monogeneans of the family Dactylogyridae with the following objectives-

- Evaluation of morphological characters.
- Evaluation of anatomical characters.
- Evaluation of molecular biological characters.

During the course of study, following host species were examined.
Table. 14. List of host examined and monogeneans collected.

<table>
<thead>
<tr>
<th>Host</th>
<th>Monogenean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mystus vittatus (Bloch, 1794)</td>
<td><strong>Bifurcohaptor indicus</strong> Jain, 1958</td>
</tr>
<tr>
<td>Cyprinus carpio (Linnaeus, 1758)</td>
<td><strong>Dactylogyrus extensus</strong> Mueller and Van Cleave, 1932</td>
</tr>
<tr>
<td>Catla catla (Hamilton, 1822)</td>
<td><strong>Dactylogyrus labei</strong> Musselius and Gusev, 1976</td>
</tr>
<tr>
<td>Ctenopharyngodon idella (Valenciennes, 1844)</td>
<td><strong>Dactylogyrus lamellatus</strong> Achmerow, 1952</td>
</tr>
<tr>
<td>Puntius sophore (Hamilton, 1822)</td>
<td><strong>Dactylogyrus longicirrus</strong> Tripathi, 1959 Gusev, 1976</td>
</tr>
<tr>
<td>Macroganthis aculeatus (Hamilton, 1822)</td>
<td><strong>Mastacembelocleidus bam</strong> Tripathi, 1959 Kritsky et al., 2004</td>
</tr>
<tr>
<td>Notopterus notopterus (Pallas, 1769)</td>
<td><strong>Notopterodiscoides notopterus</strong>, (Jain, 1952) Agrawal and Vishwakarma, 1999</td>
</tr>
<tr>
<td>Nandus nandus (Hamilton, 1822)</td>
<td><strong>Sundanonchus behuri</strong> (Agrawal and Singh, 1982) Tripathi et al., 2009</td>
</tr>
<tr>
<td>Anabas testudineus (Bloch, 1792)</td>
<td><strong>Trianchoratus kearni</strong> Agrawal and Bhatnagar, 1994</td>
</tr>
<tr>
<td>Xenentodon cancila (Hamilton, 1822)</td>
<td><strong>Xenentocleidus xenentodon</strong> Jain, 1961 Tripathi et al., 2007</td>
</tr>
</tbody>
</table>

Details of the morphological characterization are:

1. **Bifurcohaptor indicus** Jain, 1958 has been collected from **Mystus vittatus** (Bloch, 1794) from Guwahati, Assam. This is the first report of this parasite from the Brahmaputra river, Assam. The parasite of this region is similar to as those of Ganga River system. Although little morphological and morphometric differences are found in the measurements of various body parts. 28S rDNA sequence show presence of 341 base pairs (JX852710)

2. **Dactylogyrus extensus** Muller and Van cleave, 1932 has been described from **Cyprinus carpio** (Linnaeus, 1758) at Guwahati. This species is invasive and first time reported from India. It is described on the basis of morphological descriptions and morphometric analysis. The reasons of invasion of this species in India are discussed in detail. Both 18S and 28S rDNA sequences were worked out and submitted to NCBI with accession number JQ926197 and JQ926197.
3. *Dactylogyrus labei* Musselius and Gussev, 1976 was collected from *Catla catla* (Hamilton, 1822) from Guwahati, Assam. This parasite has two forms, typical and atypical. The investigator got atypical form of *Dactylogyrus labei*. Originally this parasite was reported from the host *Labeo rohita* (Hamilton, 1822) but during this study, the fish *C. catla* was found harbouring with this parasite. The parasite studies exhibits minor variations in several body features besides measurements. The investigator found that this is the case of host switching which is discussed in detail. Besides this, taxonomic validation has also been made using 28S rDNA sequence submitted to NCBI (JX566720).

4. *Dactylogyrus lamellatus* Achmerow, 1952 was collected from the type host, *Ctenopharyngodon idella* (Valenciennes, 1844) at Guwahati, Assam. This parasite is also invasive and first time reported from India. The probable reasons for the invasion are discussed in detailed beside the morphology and molecular biology. Both 18S and 28S rDNA regions were used for the study with submissions to GenBank (JQ926199 and JQ926200).

5. *Dactylogyroides longicirrus* (Tripathi, 1959) Gusev, 1976 has been collected from the gills of *Puntius sophore* (Hamilton, 1822) from locality Guwahati, Assam. This species differs from all other species by having large sized dorsal anchors with a well sclerotised thick ventral bar. Besides morphology, 18S rDNA sequences (KC685371) was also used in this study.

6. *Mastacembelocleidus bam* (Tripathi, 1959) Kritsky *et al.*, 2004 was collected from the type host, *Macrognathus aculeatus* (Bloch, 1786) at Guwahati, Assam. Some morphological variations have been observed in the specimens at the disposal of writer besides measurements. The morphology are discussed in detail. Apart from morphology, 28S rDNA sequence of 324 base pairs (JX987076) has also been used for this study.

7. *Notopterodiscoides notopterus* (Jain, 1952) Agarwal and Vishwakarma, 1999 collected from the gill filaments *Notopterus notopterus* (Pallas, 1769) at Guwahati, Assam and is characterized by size of anchors, shape of male copulatory tube, accessory piece and vaginal armament. 28S rDNA sequence, 337 base pairs was also used to validate the taxonomy of this species (JX444912).

8. *Sundanonchus behuri* (Agrawal and Singh, 1982) Tripathi *et al.*, 2009 has been collected from *Nandus nandus* (Hamilton, 1822), the type host but from different locality, Guwahati,
Assam. The variations recorded are discussed in detail. 28S rDNA has also been used in this study containing 310 base pairs (JX444913).

9. *Trianchoratus kearni* Agrawal and Bhatnagar, 1994 has been collected from gills of *Anabas testudineus* (Bloch, 1792) in Guwahati, Assam. This species is characterized on the basis of shape of cirrus and its accessory piece. Besides this, 28S rDNA comprising of 355 base pairs (JX987077) has also been studied.

10. *Xenentocleidus xenentodoni* (Jain, 1961) Tripathi *et al.*, 2007 collected from gills of *Xenentodon cancila* (Hamilton, 1822) in Guwahati, Assam. Morphologically, the present form is similar to the same species reported from Ganga River system. Besides morphology 28S rDNA having 359 base pairs has also been used to validate the species (JX987075).

Besides describing morphology, the investigator also explored the possibility of using DNA sequences for taxonomic purposes. Species specificity of the nucleotide sequence of small and long subunit of rDNA region has been used. The rDNA markers 18S and 28S was found to be very conservative and these segments has also been successful to detect and describe new species.

The 18S and 28S region of the rRNA gene has been found to be useful in molecular phylogeny. Molecular characteristics are more useful than morphology to demonstrate the evolutionary relations in a quantitative manner between specimens, populations and species. These observations in addition to the morphological variability will solve the problems concerning the validity of the species and genera of monogeneans. All phylogenetic trees were constructed by MEGA 5.0. Since the substitution rate among sites are not uniform, Kimura-2-parameter distance was used, Neighbour joining and Maximum Parsimony trees were tested by 1,000 bootstrap repeats. Possible use of these tools in monogenean systematics and phylogeny has been discussed in detail.
The thesis is divided into following chapters:

1. INTRODUCTION
2. HISTORICAL REVIEW
3. MATERIAL AND METHODS
   - Sampling site
     - Parasite collection and Morphological analysis
   - Molecular biological study of parasites
4. RESULTS AND DISCUSSION
   4.1. Bifurcohaptor indicus Jain, 1958
   4.2. Dactylogyrus extensus Mueller and Van Cleave, 1932
   4.3. Dactylogyrus labei Musselius and Gusev, 1976
   4.4. Dactylogyrus lamellatus Achmerow, 1952
   4.5. Dactylolgryoides longicirrus (Tripathi, 1959) Gusev, 1976
   4.6. Mastacembelocleidus bam (Tripathi, 1959) Kritsky et al., 2004
   4.7. Notopterodiscoides notopterus (Jain, 1952) Agrawal and Vishwakarma, 1999
   4.8. Sundanonchus behuri (Agrawal and Singh, 1982) Tripathi et al., 2009
   4.9. Trianchoratus kearni Agrawal and Bhatnagar, 1994
   4.10. Xenentocleidus xenentodon (Jain, 1961) Tripathi et al., 2007
5. GENERAL DISCUSSION
6. SUMMARY
7. REFERENCES
As whole, the thesis covers 186 pages. All the figures are original, drawn by the investigator for which he is solely responsible. The thesis is illustrated with the help of 41 figures and 14 tables. A list of 499 references having direct bearing with the work are given at the end of the thesis. Besides this, a number of journals, periodicals and books were also consulted. The type and paratype slides are deposited in the museum of Department of Zoology, Chaudhary Charan Singh University, Meerut.
Monogenean (Platyhelminthes) richness in Northeast Indian region, Assam: A component of forgotten biodiversity

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Abstract. This study is the first attempt, aiming to assess the diversity of monogenean species on the freshwater fishes of Northeast region, Assam, India. A total of 14 putative species of monogeneans was recognized, belonging to 10 genera and one family. The monogenean fauna from India is always repeated with information on diversity in fishes but surprisingly except this region. A very little information exists on the monogenea fauna of Northeast India and more specifically on those of major River system Brahmaputra, Assam. From this important body due to paucity of monogenean studies in Assam, the present investigation has been started. The highest diversity and abundance of parasites were found for the family Dactylogyridae and also found that this is the best known region for further work on monogenean diversity. Considering the great importance of fishes in the ecosystem and in order to improve our current knowledge on the diversity of freshwater monogenean species this study was made.

Keywords: Monogenean; Assam; Brahmaputra; Biodiversity; India.

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Introduction

The Monogenea is a class of flatworms parasitic mostly on external surfaces and gills of freshwater fishes. Monogeneans are obligate parasites of aquatic and semi-aquatic organisms because they are unable to withstand desiccation (Bychowsky, 1957). Fish forms the main host for the majority of the known monogeneans (Euzet and Combes, monogeneans in Indian subcontinent has not been documented completely. Surprisingly, negligible information exists on monogenean fauna of Northeast India and more particularly from major River system Brahmaputra, Assam. Approximately 4000 species of Monogenea up to 25000 species are predicted to exist (Whittington, 1988). But in India no study on fishes of this region for monogenean parasites has been carried out till now. The monogenean
taxa from Assam are awaiting their discovery and formal description in biodiversity studies. Due to this paucity of monogenean studies in Assam state there is a lack of data about their prevalence of infection on piscine hosts. Currently, there is no data available on monogeneans diversity for this area regarding their diversity, ecology or any other parameter.

With an aim to evaluate records of the monogeneans in this region the present study was conducted. Despite this, the knowledge of the monogeneans in the Northeast region has improved our knowledge about the diversity of present study was to assess monogeneans biodiversity of this area as well as to identify how wide of species diversity is exists in this area.

Materials and methods

During the study, the hosts were caught from the river Brahmaputra on the site Guwahati confirmation their identification by keys (Dey, 1958; Misra, 1959; Srivastava, 1968) and ichthyologists, the fish were killed by a sharp blow on the top of the head and dissected. Monogeneans were collected from the gills of fishes, according to the method suggested by Malmberg (1970). Parasites were processed for morphological examination (Malmberg, 1970). Identification of the monogeneans were made with the help of morphology of the haptoral hard parts and copulatory complex. The slides of parasites have been deposited in the museum of the Department of Zoology, Chaudhary Charan Singh University, Meerut, U.P., India, under voucher numbers listed in table (table 1).

Results

Overall, 14 putative species of monogeneans were recognized. These belonged to 10 genera and 1 family of which they have been identified to species level (table 1). All species of monogeneans were found belong to the family

one of the most specious family of the class Monogenea. The Dactylogyridae (Bychowsky, 1937) is the most diverse order within the

Polyonchoinea (Bychowsky, 1937). Within this order Bychowsky (1937) for the first time included the family Dactylogyridae (Bychowsky, 1933).

The family Dactylogyridae were represented by 14 species in 10 genera (Bifurcohaptor Jain, 1958; Cornudiscoides Kulkarni, 1969; Dactylogyrus Diesing, 1850; Dactylogyroides Gusev, 1963; Mastacembelocephalus Kritsky et al. 2004; Notopterodiscoides Lim and Furtado 1986; Malayano discoides Lim and Furtado 1986; Sundanochus Lim and Furtado 1985; Trianchoratus Price and Berry 1966; Xenentolecidus Tripathi et al. 2007) presented species in the table (table 1). From this important major River system, it seems likely that monogenean diversity is more widely spread than previously thought. The class successful group in this region as indicated by their high diversity of parasites. This study evidently confirms the high number of parasites on the basis of availability. It is seen that these parasites are successfully established on the fish fauna of this major River system, may be due to some favorable conditions shown by the host and the environment. Thus, there is a urgent need to focus attention on study of Monogenea in this region, to control the diseases spread by these parasites and to the successful production of
fishery resources in the state because in this region the fishes are the main supplement protein requirement for the poor.

Discussions

After this study, it should be noted that this study is not complete and more intensive survey is still needed because monogenean parasites fauna along with their sensitive host i.e., fish causes a rapid environmental deterioration. The present study represents a first attempt to increase the knowledge of monogenean parasites in the Guwahati, Assam. It aims to establish the base for future discussion of the diversity of freshwater monogenean in the northeast region which is currently biased by lack of information and lack of workers in this field.

The present biodiversity investigation aims to parasites in northeast region, Assam, which is untouched region in India regarding these parasites. The family Dactylogyridae of Monogenea class is found to be the highest abundance and diversity which shows the successful establishment of these parasites on their respective hosts. This study improves the current knowledge of freshwater monogenean distribution as well as to clarify their biodiversity that represents regional and global benefits for researchers worldwide.

<table>
<thead>
<tr>
<th>Monogenean species</th>
<th>Fish Hosts</th>
<th>VoVoucher No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Order Dactylogyridae</td>
<td>Mystus attatus (Bloch)</td>
<td>HS/monogenea/2012/06</td>
</tr>
<tr>
<td>Family Dactylogyridae</td>
<td>Mystus attatus (Bloch)</td>
<td>HS/monogenea/2012/13</td>
</tr>
<tr>
<td>Corinaeidae proaximus Gusev, 1976</td>
<td>Cirrhinus cirrhosus (Bloch)</td>
<td>HS/monogenea/2012/14</td>
</tr>
<tr>
<td>Dactylogyrae sanabelis Gusev and Musselius, 1975</td>
<td>Cirrhinus cirrhosus (Bloch)</td>
<td>HS/monogenea/2012/02</td>
</tr>
<tr>
<td>Dactylogyrae sanabelis Mueller and Van Cleave, 1932</td>
<td>Cirrhinus cirrhosus (Linnaeus)</td>
<td>HS/monogenea/2012/05</td>
</tr>
<tr>
<td>Dactylogyrae sanabelis Habe and Gusev, 1976</td>
<td>Cirrhinus cirrhosus (Linnaeus)</td>
<td>HS/monogenea/2012/01</td>
</tr>
<tr>
<td>Dactylogyrae sanabelis Gusev, 1976</td>
<td>Cirrhinus cirrhosus (Linnaeus)</td>
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<tr>
<td>Dactylogyrae sanabelis Habe and Gusev, 1976</td>
<td>Cirrhinus cirrhosus (Linnaeus)</td>
<td>HS/monogenea/2012/01</td>
</tr>
<tr>
<td>Mastacembeloidea baum (Tripathi, 1959)</td>
<td>Macrolepidotus aculeatus (Hamilton)</td>
<td>HS/monogenea/2012/07</td>
</tr>
<tr>
<td>Kritsiv et al. 2004</td>
<td>Macrolepidotus aculeatus (Hamilton)</td>
<td>HS/monogenea/2012/07</td>
</tr>
<tr>
<td>Notoperoridicola notoperus (Jain, 1952)</td>
<td>Notoperoridicola notoperus (Pallas)</td>
<td>HS/monogenea/2012/03</td>
</tr>
<tr>
<td>Agranawal and Vishvakarma 1996</td>
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</tr>
<tr>
<td>Sundarapuricola behari (Agrawal and Singh 1982)</td>
<td>Notoperoridicola notoperus (Pallas)</td>
<td>HS/monogenea/2012/03</td>
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<tr>
<td>Tripathi et al. 2009</td>
<td>Notoperoridicola notoperus (Pallas)</td>
<td>HS/monogenea/2012/03</td>
</tr>
<tr>
<td>Triplicataurus keorn Price and Berry 1966</td>
<td>Anabas testudineus (Bloch)</td>
<td>HS/monogenea/2012/10</td>
</tr>
<tr>
<td>Xenentodon xenentodon (Jain, 1961)</td>
<td>Xenentodon concilii (Hamilton)</td>
<td>HS/monogenea/2012/09</td>
</tr>
</tbody>
</table>

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We are thankful to the Head of the Department of Zoology, Chaudhary Charan Singh University, Meerut, India for providing laboratory facilities.
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Xerentodon cancila (Teleostei: Beleniformes: Belonidae) in India, with proposal of
Xerentocheilus n.g. (Monogenoidea: Dactylogyridae). Comp. Parasitol. 74:260-263.

Dactylogyridae: Sundanostochidae) from the gills of the freshwater Gangetic leaf fish, Nonius

Whittington L.D. 1988. Diversity “down under”: a prediction of monogenean biodiversity world-
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 joining 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 use 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 haptorial 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 at -20°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), 0.4 mM 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.

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 Gusev [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 \( \Delta G = -290.40 \) 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.

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 Dactylogyrus 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 Dactylogyrus 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.

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 Dactylogyrus 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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Molecular Characterization of Dactylogyroides tripathii (Monogenea, Dactylogyridae) Using Long Subunit rDNA from North East Region of India

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Introduction

Dactylogyroides was proposed by Gussev, 1963 for the worms previously described under the genus Dactylogyrus Diesing, 1850 viz., Dactylogyroides tripathii (Tripathi, 1959) Gussev, 1973 from Puntius ticto from River Brahmaputra, Guwahati, Assam, India. These are the parasites of freshwater cyprinids. Gussev (1963) differentiated the genus from Dactylogyrus in having anchors with their points directed towards each other, the dorsal bar usually double or single with a different degree of separation into two parts. On this basis Dactylogyrus tripathii (Tripathi, 1959) Gussev, 1973 was transferred to the new genus Dactylogyroides Gussev, 1963.

The use of molecular tools for the identification of parasites has become commonplace. The nuclear rDNA gene regions have also been used extensively in the study of phylogeny at several different taxonomic levels. So far, in platyhelminth systematics, rDNA genes, have been used successfully (Simková et al., 2006; Lee et al., 2007; Chiary et al., 2013) with 28S rDNA, in particular, to estimate the relationships existing among the Platyhelminthes (Olson et al., 2003). Monogenean sequences of partial 28S rDNA have been used successfully to study phylogenetic relationships (Mollaret et al., 2000a; 2000b; Justine et al., 2002; Olson, Littlewood, 2002; Whittington et al., 2004; Wu et al., 2008; Simková et al., 2006; Lee et al., 2007; Chaudhary, Singh, 2012; Verma et al., 2012).

The aim of the present study is to characterized Dactylogyroides tripathii found infecting the fish P. ticto from River Brahmaputra, Guwahati, Assam, India using partial sequence of the 28S rDNA.
Material and methods

Monogeneans were collected from the gills of Puntius ticto Hamilton, from River Brahmaputra at the site Guwahati (26°11' N and 91°44' E). After the fish identification, they were killed by a sharp blow on the top of the head and dissected. Methods of collection, extraction, amplification and sequencing of monogeneans were followed from Singh and Chaudhary (2010) using specifically designed primer (forward, 5'-TCTAGTAAACGGCGAGTGACG-3') and (reverse, 5'-GGTGAAGGCTCTACCTCAGC-3'). The specimens of D. tripathii have been deposited in the Museum, Department of Zoology (voucher number HS/molongenea/2012/11), Chaudhary Charan Singh University, Meerut (U. P.), India. The obtained sequence that included the partial 28S sequence was submitted to GenBank under accession number JX993982.

For phylogenetic analysis, GenBank was first queried to retrieve 28S sequences from monogeneans and then aligned using ClustalW implemented in MEGA 5.05 (Tamura et al., 2011). Phylogenetic trees were reconstructed using Neighbour Joining and Minimum Evolution methods by MEGA 5.05. In reconstructing the NJ tree, the Kimura two-parameter model was used to estimate the distances. Kimura's two-parameter model (1980) corrects for multiple hits, taking into account transitional and transversional substitution rates, while assuming that the four nucleotide frequencies are the same and that rates of substitution do not vary among sites. Robustness of the inferred phylogeny was assessed using a bootstrap procedure with 1,000 replications.

Results

A 733 bp fragment of the 28S rDNA sequence was amplified from the specimens of D. tripathii. Nucleotide frequencies (percent) were T = 215, C = 144, A = 167, and G = 207. Phylogenetic tree of long subunit sequences showed similar grouping using the different methods employed (fig. 1, 2). The phylogenetic reconstructions inferred from analyses of the 28S rDNA sequences showed great resolution for the species of the monogeneans. This species shows nucleotide identity of 90 % with the different species of genus Dactylogyrus from which it was originally differentiated by Gussev (1963) including one species of same genus viz., Dactylogyroides longicirrus (91 %).

Fig. 1. Neighbor joining (NJ) tree of Dactylogyroides tripathii showed its phylogenetic relationship with other species of monogeneans; bootstrap values are indicated in the nodes.

Рис. 1. Дендрограмма по методу связывания ближайших соседей (NJ — метод) Dactylogyroides tripathii, показывающая ее эволюционную связь с другими видами; значения буферного значения приведены в узлах.
Delimiting species of Dactylogyroides monogeneans is often difficult, owing to their limited morphological characters, and it may have resulted in a gross estimation of the true number of species. About fourteen species of this genus have been described from India on the basis of morphological analysis and from these till now only one species viz., *D. longicirrus* (Tripathi, 1959) Gussev, 1973 has been characterized at molecular level (Singh, Chaudhary, 2010).

Gussev (1973) recorded four related monogeneans of genus Dactylogyroides from the different piscine hosts Puntius stigma, Barbus mahecola and B. dorsalis. But the original description of these specimens was not completed and the author (Gussev, 1973) was not sure about the taxonomic status of these worms. So, the taxonomic status of these species viz., Dactylogyroides tripathii f. dorsalis, D. tripathii f. filamentosi, D. tripathii f. mahecola and D. tripathii f. stigma suffers from serious lapses.

Moreover, Dubey et al. (1997) redescribed Dactylogyroides tripathii (Tripathi, 1959) Gussev, 1973 from Puntius sophore at Raipur. Agrawal et al., (2002) made a comprehensive review of Indian species of Dactylogyroides Gussev, 1963. The evaluation of morphological criteria for phylogenetic and taxonomic studies of the monogeneans seems to be the most controversial area (Desévesiès, 2001; Wu et al., 2006, 2007; Chaudhary, Singh, 2012). 28S region analyses in this study revealed that this gene is a good phylogenetic marker for inferring relationship between closely related species. DNA based identification used during this study has enabled the molecular characterization of *D. tripathii*.
In the present study, D. tripathii shows the close similarity with another species of same genus viz., D. longicirrus (Tripathi, 1959) Gussev, 1973 and after that with various species of genus Dactylogyroides from which it was differentiated. The tree topologies derived from the phylogenetic analysis inferred from 28S rDNA data depicted that both the genus Dactylogyroides and Dactylogyrus, as genetically, closely related sister taxa as they formed closely related clade (fig. 1, 2). Therefore, based on our molecular analysis results by different methods, we propose that the species D. tripathii was correctly accommodated in the genus Dactylogyroides by Gussev, 1973. This study further confirmed that 28S rDNA is useful marker for distinguishing sister genera or species and helpful in discriminating species especially when morphological differences are often difficult to determine.

In conclusion, the present identification of the D. tripathii species with 28S sequence analysis is consistent with investigations made using only traditional approaches, i.e., by morphology. The molecular study with 28S sequence is a promising tool for monogenean identification at species level. We believe that such taxonomic revisions based on molecular biology will continue with the increasing number of Dactylogyroides species for comparison and being used for molecular phylogenetic investigations in the future.

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References


The Chorionic Sculpture in Eggs of Some Noctuidae (Lepidoptera)


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