Morphometric and molecular identification of *Paradactylogyrus catlaius* (Thapar 1948) in *Catla catla* (Hamilton 1822)

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Abstract *Paradactylogyrus* are small monogenean ectoparasites found mostly on the gills of Cyprinidae and are highly host specific. Conventionally these parasites are identified by microscopic analysis. In contrast to this conventional method, molecular identification using nuclear ribosomal DNA (rDNA) provides specific, sensitive and safe detection of parasites. The present study is aimed to identify the *Paradactylogyrus* on *Catla catla* using morphological and molecular tools. Morphologically the parasite is having hamuli, marginal hooks, bars and copulatory organs which differentiate it from other monogeneans. The parasite is having unpaired central spine, tubular cirrus and extracaecal vitellaria. 28s rDNA study revealed that the species is *P. catlaius* and the sequence was submitted to Genbank (accession no. KF269531). The study suggests that morphological characters along with molecular study are essential for validating and identifying the monogeneans. This is the first published sequence of species from the genus *Paradactylogyrus* (Thapar 1948).

Keywords *Catla catla* · Monogenea · Morphometric analysis · *Paradactylogyrus catlaius* · PCR · 28s rDNA

Introduction

Catla catla is a freshwater fish belonging to the family cyprinidae and is known by the common name of catla. It is

one of the most commonly edible Indian major carp. Cyprinid fishes are the significant freshwater-cultured fish and have a high market value in fish trade. However, the main drawbacks for the extensive commercial production of this species are frequently associated with diseases including bacterial, viral and parasitic infections. However, growth in production of these species during the past decades has resulted in much greater attention being paid to problems posed by parasites and their importance for fishery leading to loss in the productivity of aquaculture. Besides direct losses caused by mortality, parasites have considerable impact on growth and behaviour of fish, their presence may also reduce marketability of fish (Crowden and Boom 1980; Lom and Dykova 1992; Williams and Jones 1994; Kumaraguru et al. 1995; Woo 1995).

Dactylogyrus (Dactylogyridae: Monogenea: Platyhelminthes) represents the most dominant genus among the Monogenea with regards to host distribution and location. These are common ectoparasites usually attached to the gills of freshwater fish of the family Cyprinidae (Gibson et al. 1996; Woo 2006). Also they are hermaphroditic in nature. Heavy infections of the parasites occur frequently in high density environments and induce lowered growth performance, loss of appetite, and high mortality in fish due to gill pathology and interference with the exchange of oxygen and ions, as well as secondary infection by bacteria, virus and fungus (Roberts 2001). Infection by *Dactylogyrus* causes lesions in the gill epithelium during attachment and these lesions are caused by the 14 marginal hooks and 2 anchors of the attachment organ. As they possess a direct life cycle, they can reach epizootic levels very quickly when hosts and parasites are confined close together (Thoney and Hargis 1991; Blazek et al. 2008).

Microscopic analysis of gill lamellae is the traditional method used to detect and identify *Dactylogyrus*

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parasites. However, *Dactylogyrus* spp. is very small (usually <1 mm) and their collection from the gills requires optical instruments. Species identification of monogeneans is based mainly on the morphology of the hard parts of their haptors and copulatory organs (Bruno et al. 2006). Even molecular techniques and DNA sequence analyses are useful for comprehending the molecular taxonomy and phylogeny of *Dactylogyrus* spp. (Shamsi et al. 2009). Since the introduction of molecular tools into taxonomy, systematic and phylogeny, many species descriptions are re-evaluated, new species are described while some are assigned new taxonomic positions (e.g. Burridge and White 2000; Jousson et al. 2000; Desdevises 2001; Lazoski et al. 2001).

The genus *Dactylogyrus* is the largest helminth genus, with more than 900 species (Neary et al. 2012). Many of its species are not yet described. A morphological identification method has been developed by Malmberg (1970) based on the hard parts of the posterior attachment organ. The classical morphological analysis is important but it may lead to an underestimation of the number of species. To resolve the problems of identification of *Dactylogyrus*, molecular methods were inspired from the molecular techniques introduced by Cunningham et al. (1995) for identification of *Gyrodactylus*. The present study aimed to identify species *Paradactylogyrus catlaius* infecting *C. catla* in Eastern India causing mass mortality of this species using both morphological and molecular characteristics. Therefore, the study is directed to have a comparative account of morphological observation and ribosomal DNA (rDNA) analysis of *P. catlaius*.

Materials and methods

Parasite collection and identification

Parasites were collected carefully from the gills of infected *C. catla* (Hamilton 1822), captured from Mahukhanda, Odisha, India and fixed in 70 % ethanol for future use. An individual parasite was drawn out from the 70 % ethanol fixed parasites, placed on a glass slide with a drop of water and stained with a mixture of 2 reagents i.e., ammonium picrate glycerine (Malmberg 1970) and 10 % SDS in a ratio of 1:1. This stain makes the parasite transparent which results in a better study of the hard parts of the haptor and copulatory organs of the parasite. A total 100 parasites were collected from the infected *C. catla* of average weight 250 g. All were observed under Nikon Eclipse 50i phase contrast microscope (0.90 dry, Japan) for the morphometric analysis of their hard parts and copulatory organs. Parasites were then identified to species level morphologically,

according to the existing keys and descriptions made by Yamaguti (1961).

DNA extraction and PCR

For genomic DNA extraction, an individual specimen was taken in a 2 ml tube from the 70 % ethanol fixed parasites. DNA was extracted from the parasite using the Qiagen DNeasy Blood and Tissue Kit according to the manufacturer's instructions. A region comprising partial 28S rDNA was amplified using the universal primers, forward (5'-ACCCGCTGAATTTAAGCAT-3') and the reverse primer (5'-CTCTTCAGAGTACTTTTCAAC-3'). Each amplification reaction was performed in a final volume of 25 µl containing 3 µl of genomic DNA extract, 10× PCR buffer, 0.4 mM dNTP, 10 pM of each primer, 3 U Taq polymerase (Geni) and Milli-Q water in a thermocycler (Veriti 96 well Thermal Cycler) under the following conditions: 2 min at 95 °C (initial denaturation), followed by 40 cycles of 1 min at 95 °C (denaturation), 1 min at 48 °C (annealing) and 1 min at 72 °C (extension) and a final extension at 72 °C for 10 min. PCR products were examined on 1.2 % agarose-TAE gels, stained with ethidium bromide and visualized under ultraviolet light.

PCR product purification and sequencing

The PCR products were purified from gels using a Minelute Gel Extraction Kit (Qiagen) according to manufacturer's instructions. The purified gel extracted PCR product was then sequenced by SciGenom Labs in India using a method based on Sanger et al. (1977). Only forward primer (5'-ACCCGCTGAATTTAAGCAT-3') was used for sequencing. Sequence similarity searching for *Dactylogyrus* was performed using the NCBI BLAST online application.

Results

Species description

Paradactylogyrus catlaius (Thapar 1948)

Order: Monogenea

Suborder: Monopisthocotylea

Family: Dactylogyridae

Subfamily: Dactylogyrinae

Genus: *Paradactylogyrus*

Species: *catlaius*

Host: *Catla catla* (Hamilton 1822)

Type locality: Mahukhanda, Khordha, Odisha, India.

Site of infection: Gills.

Morphological examination: 100 specimens

Table 1 Morphological measurements of opisthaptor hard parts of *Paradactylogyrus* sp. collected from *Catla catla* in the present study

Morphometric measurements	
Characters	Measurement (μm)
Total length (T.L)	904.41 ± 1.64
Total width (T.W)	132.70 ± 0.78
Anchor total length (A.T.L)	37.33 ± 0.28
Anchor base length (A.B.L)	28.98 ± 0.26
Anchor root (A.R)	8.34 ± 0.23
Anchor point (A.P)	27.64 ± 0.31
Anchor width (A.W)	33.22 ± 0.26
Total length of ventral bar (T.L.V.B)	56.0 ± 0.24
Total width of ventral bar (T.W.V.B)	8.09 ± 0.17
Hook total length (H.T.L)	25.23 ± 0.18
Hook base (H.B)	22.63 ± 0.18
Hook point (H.P)	2.6 ± 0.00
Whole length of copulatory organ (W.L.C.O)	134.48 ± 0.62
Vaginal length (V.L)	37.02 ± 0.30

Data expressed as mean $\pm$ standard error ($n = 100$)

Morphological description

The morphological characteristics of *Dactylogyrus* samples were compared with available *Dactylogyrus* morphology. An analysis was made from the morphological characteristics of the *Dactylogyrus* specimens. The opisthaptor hard parts of specimens were measured and the data recorded (Table 1). Based on the following facts and identification keys given by Yamaguti (1961) the parasite was identified as *P. catlaius* (Thapar 1948). The characteristic or identifying feature for which the species was described as *Paradactylogyrus* was the unpaired central spine or hook (Figs. 1, 2).

Molecular description

DNA was extracted from individual parasites and was PCR amplified. The results showed an expected PCR product of 331 nucleotides in length (Fig. 3). The PCR products were purified and then sequenced. After comparing the nucleotide sequences of the PCR products with GenBank sequences, the sequence was found to be a new one. The sequence was then submitted to GenBank as sequence of *P. catlaius* (Thapar 1948) based on its morphometric analysis under the accession no. KF269531.

Discussion

This study presents both morphological and molecular descriptions of *Dactylogyrus* species on *C. catla*. Species of the monogenean, genus *Dactylogyrus* are difficult to identify morphologically (Singh & Chaudhary 2010). The large number of species, small size (<1 mm) and the tiny

Fig. 1 Whole mount of *Paradactylogyrus catlaius* (Thapar 1948) stained with a mixture of ammonium picrate glycerine and 10% SDS in the ratio of 1:1, clearly showing the anchors, hooks, bars and copulatory organ

Fig. 2 Opisthaptor with an unpaired central spine which is the characteristic feature of genus *Paradactylogyrus*

marginal hook sickles make the identification of *Dactylogyrus* species very difficult and uncertain. Therefore for confirmation one has to rely on molecular characterization.

Fig. 3 Agarose gel electrophoresis of PCR amplification products obtained from genomic DNA of *Paradactylogyrus* sp. using 28S rDNA specific primers. Lane 1, 1 kb DNA ladder; lane 2 and 3 PCR amplified product from genomic DNA of a single parasite

Till now 5 species of *Paradactylogyrus* were identified in different locations of India by different authors. These were; *P. catlaius* (Thapar 1948), *P. bati* (Tripathi 1959), *P. thapari* (Agarwal 1980), *P. indicus* (Singh & Rastogi 2000) and *P. himalayensis* (Singh et al. 2003). The present report was based on the mortality of *C. catla* in composite fish culture from farmer's pond at Mahukhanda, Khordha, Odisha, India. It was noticed that 50 fish of *C. catla* with an average weight of 250 g was dead within 4 days. Another 450 catla showed mild infection. Based on the morphometric identification made by Thapar (1948), the species was detected as *P. catlaius*. It is interesting to note that rest of the fish 1,500 nos. belonging to species *Labeo rohita* and *Cirrhinus mrigala* were not infected and the fishes were apparently healthy. This indicated that the *P. catlaius* is more infectious to *C. catla* than *L. rohita* and *C. mrigala*.

The species have an elongate body with a length of 0.9–1 mm and width of 0.13–0.2 mm. Its anchors are very distinct connected with a single bar of 0.05–0.07 mm, 14 marginal hooklets and an unpaired central spine. Cirrus of this species is tubular whereas it is forked in case of *P. thapari*. Vitellaria mostly extracaecal as other *Paradactylogyrus* species. Vagina is tubular, winding with lateral opening.

To confirm the morphological identification, molecular characterization was carried out. The nucleotide sequence of the parasite provided in this study is the first report on 28S rDNA sequence for *Paradactylogyrus* genus. For the study, the DNA fragment amplified here is comprised of partial 28S rDNA using universal forward and reverse primers. Though the sequence shows similarity with the *Dactylogyrus* species submitted to Genbank but morphologically they are different from the *Dactylogyrus* spp. Thus, the sequence was described as the nucleotide sequence of *P. catlaius* (accession no. KF269531).

Analyses of 28S rDNA region revealed that this gene is a very prominent molecular marker for inferring confirmation morphological study. This study can be useful for creating the sequence of other *Paradactylogyrus* species, further it will again be helpful in establishing phylogenetic relationship. Also this method could extend the range of monogenean material available for critical analysis. The results obtained from this microscopic study were confirmed by sequence analysis. A combination of morphological description with a molecular technique appears to be the complete practice for characterizing *Paradactylogyrus* species. As there are few *Paradactylogyrus* spp. in Indian subcontinent as well as worldwide and no molecular identification is available, the present study would provide a basis for species identification of *P. catlaius* for future study.

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