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Molecular characterization of two non-native species of *Dactylogyrus* (Monogenea: Dactylogyridae) recovered from introduced hosts in India

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Abstract

Freshwater fish from the Guwahati in River Brahmaputra, Assam, India were screened for infection with Monogenea. The recovered parasites were diagnosed by PCR and sequencing the 18S and 28S rDNA subunit regions. Comparison of the obtained nucleotide sequences with sequences in GenBank identified the specimens as *Dactylogyrus extensus* Mueller and Van Cleave, 1932 and *D. lamellatus* Achmerow, 1952, recovered from *Cyprinus carpio* and *Ctenopharyngodon idella* (Cypriniformes: Cyprinidae) respectively. The obtained sequences differed by only 2-3 nucleotides from the sequences deposited in GenBank. The presence of *D. extensus* and *D. lamellatus* in India can result from the introductions of their respective host carrying their parasites with them.

Key words: invasive species, *Cyprinus carpio, Ctenopharyngodon idella*, India

Introduction

A major risk associated with introductions of fish is the transmission of their parasitic fauna to native fish host that may be highly susceptible to infection (Kirk 2003; Taraschewski 2006; Kelly et al. 2009). The transmission of non-native parasites to new geographical areas can have serious effects on populations of native species. A classical example is the invasive monogenean *Gyrodactylus salaris* Malmberg, 1957 that has reduced the average density of salmon parr in infected rivers in Norway and caused losses to fisheries industries and tourism (Bakke et al. 2007; Harris et al. 2011). In addition, threats by non-native monogenean parasites has recently been documented in various studies (Christison and Baker 2011; Parker et al. 2011; Ondrackova et al. 2011; Ellender and Weyl 2014). The ecological impact of non-native monogenean parasites is particularly poorly studied in many geographical regions including India, and such studies are urgently needed.

The cyprinids *Cyprinus carpio* Linnaeus, 1758 (common carp) and *Ctenopharyngodon idella* (Valenciennes, 1844) (grass carp) was introduced to India many years back, to enhance the performance of seed production for pond culture and for stocking in lakes and reservoirs. *C. carpio* and *C. idella* have gained entry into various reservoirs either through accidental or deliberate introduction. Current Indian populations of *C. carpio* originate from two introductions to India; one in 1939 ("German" strain) and the second in 1957 ("Bangkok" strain) (Jhingran 1991). *Cyprinus carpio* was introduced to India for aquaculture, due to its easy breeding, omnivorous feeding habits, warm water adaptability, good growth and hardy nature. *C. idella* was introduced to India in 1959 by import from Hong Kong and Japan. After this unauthorized introduction, this fish became popular among the aquaculturists, proved to be a boon in aquaculture and acted as an instrument for yield optimization from ponds.

In India, the monogenean fauna of the Northeast region is unexplored. *Dactylogyrus* Diesing, 1850 is a genus of monogeneans that are highly specific to freshwater fish of Cyprinidae and includes more than 900 nominal species (Gibson et al. 1996). It includes a group of ectoparasites of fish and is recognized by a four-lobed head with four

eyespots. They are mainly morphologically identified on the basis of male copulatory organ and the haptoral parts like anchors, bars and hooks (Gussev 1985). It is a species rich genus and identified solely on the basis of morphological features is sometimes problematical for very closely related species.

Molecular analyses provide an alternative for identification of monogenean species. Most studies have been established using molecular markers of 18S and 28S region of ribosomal DNA to identify and distinguish monogenean species (see e.g. Chisholm et al. 2001; Desdevises 2001; Simkova et al. 2004; Wu et al. 2007; Chaudhary and Singh 2012, 2013). For *Dactylogyrus* species several studies have been done using molecular analyses (Simkova et al. 2004, 2007; Wu et al. 2007). The present study describes the results of a parasitological investigation of *C. carpio* and *C. idella* in India with focus on their monogenean parasites.

Methods

The two *Dactylogyrus* species included in this study were taken from the two fish species namely common carp (*Cyprinus carpio*) and grass carp (*Ctenopharyngodon idella*). About 20 fish from each species were caught from the river Brahmaputra with a local fish grocer present at the site Guwahati (26° 11′ 0″ N; 91° 44′ 0″ E), Assam, India. Immediately after captured, fish were killed by a sharp blow to the head, dissected and monogeneans collected from gill filaments. Morphology and molecular study of parasites were performed as described by Chaudhary and Singh (2013). The slides have been deposited in the museum of the Department of Zoology (voucher number HS/Monogenea/2012/01 and HS/Monogenea/2012/02), Chaudhary Charan Singh University, Meerut, UP, India.

For this study, primers were designed based on the sequence of 18S (forward, 5'-CGGTTG CAATTTTTATGTGG-3' and reverse, 5'-GAGTGATCCACCACTTGCAG-3') and 28S (forward, 5'-TCTAGTAACGGCGAGTGAACG-3' and reverse, 5'-GGTGGAAGGTCTACCTCA GC-3') using the software Primer3 (Rozen and Skaletsky 2000). Each amplification reaction was performed in a final volume of 25 µl containing 3 µl of lysate, 10x PCR buffer, 1U Taq polymerase (Biotools), 0.4mM deoxyribonucleotide triphosphates (dNTP) and 10 pM of each primer pair in a thermocycler (Eppendorf Mastercycler personal; Eppendorf). PCR amplification was performed using the following protocol: after an initial denaturation at 94°C for 3 min, 35 cycles at 94°C for 30 sec (denaturation), 55°C for 18S and 59°C for 28S for 45 sec (annealing), 72°C for 1 min (elongation) with a final elongation at 72°C for 10 min. 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 PurelinkTM Quick Gel Extraction Kit (Invitrogen) following the manufacturer's instruction. 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.

A BLAST N search (Zhang et al. 2000) of the 18S and 28S rDNA sequences was performed and the best hits were retrieved.

Results

During our parasitological investigation of Brahmaputra River near Guwahati, Assam, India, monogeneans belonging to the genus *Dactylogyrus* were found. Microscopic analysis revealed that *C. carpio* and *C. idella* were infected with two *Dactylogyrus* species respectively. Based on morphological features of the shape of the anchors, connective bar and the shape of the marginal hooks, the specimens most closely resembeled *D. extensus* and *D. lamellatus* respectively.

The selected rDNA regions were successfully amplified and sequenced from of *D. extensus* and *D. lamellatus* specimens. Sequences are deposited in GenBank with accession numbers JQ926197, JQ926198 (*D. extensus*) and JQ926199, JQ926200 (*D. lamellatus*). The 18S sequence sizes were 741, 750 base pairs and for 28S they were 730, 737 base pairs respectively. The BLAST N search showed that the obtained 18S sequences of *D. extensus* and *D. lamellatus* had no difference in K2P-distance to sequences from other geographical isolates. The 28S rDNA sequences, however, showed a difference of 0.047 (K2P-distance) for *D. extensus*, while *D. lamellatus* showed a difference of only 0.002.

Discussion

The results from the present study showed the presence of *D. extensus* and *D. lamellatus* in India. These parasites might have been introduced to India, long time ago together with their respective

hosts, but remained unrecorded due to a lack of studies. The *Dactylogyrus* monogenean parasites, *D. extensus* Mueller & Van Cleave, 1932 and *D. lamellatus* Achmerow, 1952 were recorded from the gills of *C. carpio* and *C. idella* respectively. Identification was first by examination of the copulatory organ and the haptoral parts. Because the morphological studies alone may be not sufficient for identification of *Dactylogyrus* species, additional molecular analyses were performed to allow an accurate identification. In the present study, the ribosomal DNA sequence of the 18S and 28S were used and the specimens identified as *D. extensus* and *D. lamellatus*.

Introduction of non-native invasive species is recognized as one of the greatest threats to ecological well-being (Mooney and Hobbs 2000; Pimentel et al. 2000). For biodiversity and ecosystem functioning, non-native species represent a considerable threat as well as a massive economic burden (Cox 2004; Pimentel et al. 2005). The introduction of any fish species for food, restocking, ornamental and aquaculture purposes can represent a threat to freshwater ecology. These introduced organisms may become established in the wild and have a large impact on native species (Torchin et al. 2002; Lymbery et al. 2014). A range of dynamic interactions between monogeneans and fish hosts are responsible for host finding, host specificity and host immunity (Buchmann and Lindenstrøm 2002). *Dactylogyrus extensus* and *D. lamellatus* have been recorded from much of South East Asia, Western Asia and Europe (Te 1989; Simkova et al. 2004; Wu et al. 2005; Shamsi et al. 2009; Borji et al. 2012; Mhaisen et al. 2011). The higher abundance of these parasites in this region confirms that not only hosts, but also their parasites have easily adapted to the new geographical area. Moreover, species of *Dactylogyrus* have high species intensity on the gills of cyprinid fishes and introduced infected individuals may bring all or part of their native parasites with them. It also confirms the potential for introduced fish to introduce their parasitic fauna into areas outside their geographical region (Kennedy 1994; Copp et al. 2005; Gozlan et al. 2006).

Despite the awareness about invasive species, research on the invasive parasites, impact of fishes in India is still in its infancy. This finding recorded non-native monogeneans and hypothesizes that the entries of these species might be possible through fish translocations into India. The number (>70) of parasites present showed that these non-native species have the ability to flourish and colonize within this range and could be affecting the species-rich ecosystems. This represents a considerable threat to aquatic biodiversity and fishery development of country within a relatively short time scale. Therefore, detailed monitoring of establishment, expansion and impact of non-native fauna are urgently needed for proper management and possible eradication in the Indian region. To prevent new parasite introductions in this region, there is a need to emphasize monitoring of fish movements and to strengthen the effort to protect freshwater fisheries. The findings in the current study are important in that it document the spread of these species into non-native habitats. Future host colonizations and their effects may thus be better understood and managed for these parasites and their hosts.

In conclusion, this study reports for the first time the presence of non-native monogenean parasites in India, emphazising the need for more studies in the future.

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