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Identification of *Thaparocleidus caecus* (Mizelle & Kritsky, 1969) (Monogenea: Dactylogyridae) using morphological and molecular tools: a parasite invasion in Indian freshwater

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Abstract

The freshwater iridescent shark, *Pangasianodon hypophthalmus* (previously *Pangasius sutchi*) (Pangasiidae), was found in fish markets in Meerut, Uttar Pradesh, India. Parasitological examination of *P. hypophthalmus* revealed the presence of a non-native monogenean parasite (genus *Thaparocleidus*) found on gill filaments. Initial morphology and morphometrics of the parasite showed characteristics of *Thaparocleidus caecus*, known from Southeast Asia. Genetic analyses of the partial 28S rDNA confirmed the parasite as the first record of *T. caecus* in India provided.

Key words: biological invasions, non-native, rDNA, Monogenea, India

Introduction

In the context of biological invasions, scientists are increasingly aware of the devastating effects of invasive species on native communities (Cunningham et al. 2003; Torchin and Lafferty 2009; Tompkins et al. 2010). The establishment of introduced species is progressively invading world faunas with severe impacts on native species (Cunningham et al. 2003; Taraschewski 2006; Marcogliese 2008). Exotic species cause tremendous economic loss, modification of ecosystem functioning and threaten native systems (Pimentel et al. 2000; Meyerson and Reaser 2003).

Pangasianodon hypophthalmus (Sauvage, 1878) is a native fish (not a shark) to the Mekong basin, as well as the Chao Phraya River, Southeast Asia where it is intensively cultivated for food. According to Chattopadhyay et al. (2002) *P. hypophthalmus* was introduced into the farming system in Bengal, India, from Thailand through Bangladesh in 1994–1995. In a more recent study however, Singh and Lakra (2012), suggest that this fish was not introduced into Bengal until 1997 from Bangladesh. Much work has been carried out on the induced spawning and reproductive habits of this species in India to maximize productivity and provide best economic profit. It is now a successfully established species in India's freshwater (Chattopadhyay et al. 2002; Chand et al. 2011). Unfortunately, it seems the fish may have been introduced in the 1990s, without examination of its parasitic fauna (Lakra and Singh 2010; Singh and Lakra 2012). The occurrence of the parasite on *P. hypophthalmus* in this region was unexpected.

This is a new record of a *Thaparocleidus* species, *Thaparocleidus ceacus* (Mizelle & Kritsky, 1969) on *P. hypophthalmus* in India. Species determination was based on both morphological and molecular data.

The main objective of this study is to bring about the awareness of the presence of this non-native host and parasite species, to call on increased vigilance on its expansion range and to highlight the need for monitoring potential impacts.

Methods

Observations were noted of a non-native fish species in the local markets in Meerut (29º01'N, 77º45'E), Uttar Pradesh, India. Specimens were brought to the Laboratory, Chaudhary Charan Singh University, Meerut and identified as *Pangasianodon hypophthalmus* (previously *P. sutchi*). Gill filaments were examined for parasites and found to be heavily infested. Morphological and molecular studies of the parasites were performed according to Chaudhary and Singh (2012). All the measurements were taken in micrometers (µm). A BLAST search of the 28S ribosomal DNA sequences was performed, best hits retrieved, and appropriate homologues sequences aligned using the Clustal W multiple sequence alignment program (Thompson et al. 1994). Phylogenetic analysis was carried out using the Maximum Parsimony (MP) Method by software Molecular Evolutionary Genetics Analysis version 5 (MEGA5) (Tamura et al. 2011). The robustness of inferred branches was assessed using a bootstrap procedure with 1,000 replications. The 28S rDNA sequence of *T. caecus* was deposited in GenBank under the accession number KF361477. Whole mount of specimen slides of parasite have been deposited in the museum of the Department of Zoology (Voucher number HS/Monogenea/2013/14), Ch. C.S. University, Meerut, U.P., India.

Results

Thaparocleidus caecus (Mizelle & Kritsky 1969) Gussev 1978 (Figures 1–6) was identified on the gill filaments of its piscine host *Pangasianodon hypophthalmus* (Sauvage, 1878). Infestation of this congeneric parasite was significant.

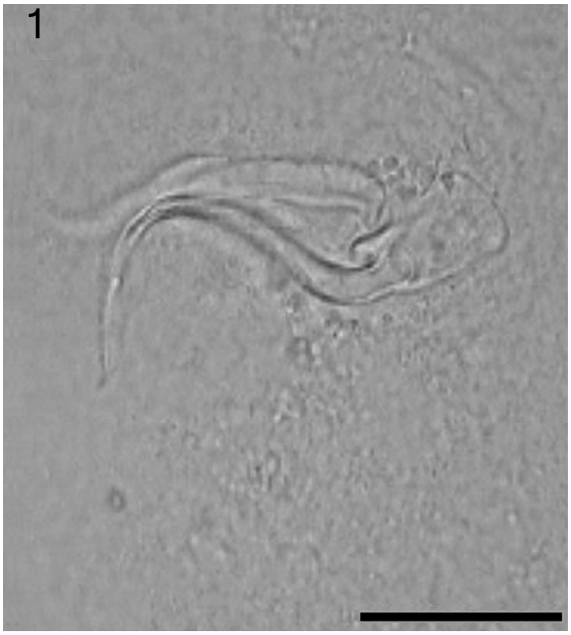
T. caecus was identified as having an elongated body with four granulated eyespots and a circular pharynx. Haptoral armature which is the chief posterior attachment organ consisted of both dorsal and ventral anchors, along with bars and marginal hooklets. Dorsal anchors were long with short, stumpy outer roots and recurved points. A triangular patch, ending in a spine was found at the base of the dorsal anchors. Ventral anchors were present with underdeveloped roots and a broad fenestrated base and recurved points. Prominent filaments were present in ventral and dorsal anchors. Dorsal bars are relatively straight with enlarged ends while ventral bars are flattened v-shape. The 14 marginal hooklets were morphologically similar. The male copulatory organ consisted of a tube and an accessory piece. The tube is sigmoid in shape and the accessory piece funnel like at the proximal end and spined at the distal end. Both tube and accessory piece articulated at the cirrus base. Anatomical measurements are given in Table 1.

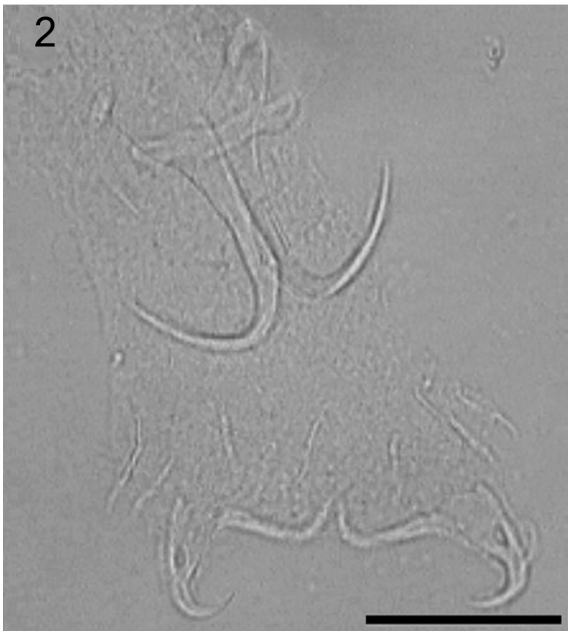
Based on the haptoral armature and copulatory complex, the specimens of *T. caecus* examined were morphologically identical to the original description of the species by Mizelle and Kritsky 1969 and later by Lim 1990. A discrepancy lies however, in relation to eye spots. In the original description by Mizelle and Kritsky 1969, "eye spots" are not present in *T. caecus*, but "eye spots granules" are present in the cephalic region in the Indian specimens examined. According to Lim 1990, it may only be a matter of terminology whether eye spot granules are considered eye spots. The morphometric measurements of *T. caecus*, agree closely with the original description. Slight variations in body size and haptor morphometry do occur (Table 1). These morphological differences may be due to geographical distribution, where environmental factors may not the same. The present record of *T. caecus* is the first of its kind recorded from India.

This is the first and only 28S sequence of *T. caecus* available on the Genbank database. The 28S rDNA sequence of *T. caecus* (627 bps) did not show a very close relationship with any other *Thaparocleidus* represented in GenBank, except *Thaparocleidus* sp. BDY (EF100555) (98%; Figure 7). The 28S tree showed a better resolution within the clade for *T. caecus* (high bootstrap values of 100%). MP method showed 28S tree with a consistency index of 0.952381, a retention index of 0.941176 and a composite index of 0.932562 for all sites, and 0.896359 specifically for parsimony-informative sites. The topology of MP tree by 28S sequences capitulated the grouping of *T. caecus* and *Thaparocleidus* sp. BDY (EF100555) (Figure 7). This similarity might be revised in the future as there has been no 28S sequence for *T. caecus* species until now.

Discussion

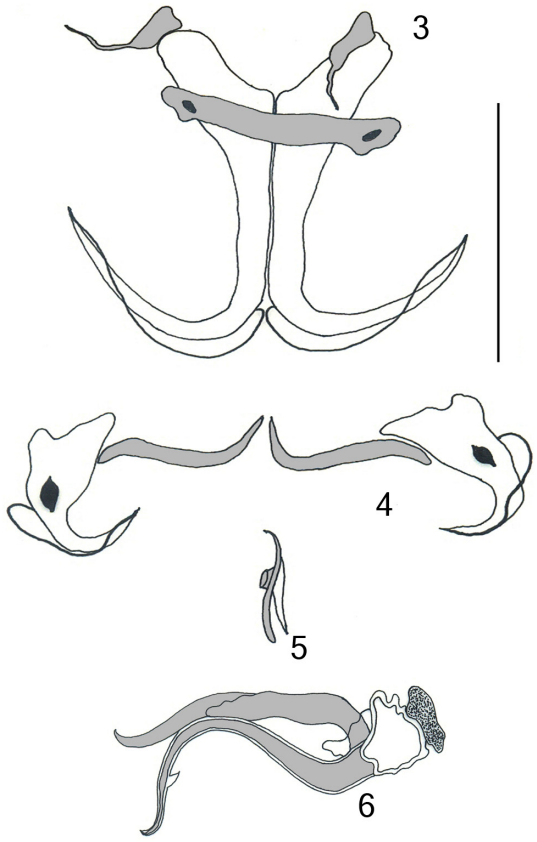
Pangasianodon hypophthalmus, a freshwater fish, is native to the Mekong River Basin in Vietnam and the Chao Phraya River in Thailand. It is widely distributed in Vietnam, Bangladesh, Indonesia and Thailand and is currently popular as a food-source in India. The species has been introduced into several ecosystems worldwide, with a high





Figures 1-2. Photomicrographs of *Thaparocleidus caecus* (**1**) Male copulatory complex, (**2**) Haptoral armature. All figures drawn to 50 µm scale.

reproduction rate, resulting in dense populations of small specimens. *P. hypophthalmus* has proven to be adaptable for intensive production in many countries (Mukai 2011), boosting overall aquaculture production (De Silva et al. 2006, 2009).



Figures 3-6. Haptoral armature of *Thaparocleidus caecus* (**3**) Dorsal anchor and dorsal bar (**4**) Ventral anchor and bars (**5**) Hook (**6**) Male copulatory organ. All figures drawn to 50 µm scale.

In India, introduced species programs are rare and many invasive pass unnoticed for a lengthy period before being detected. It has been found that *P. hypophthalmus* and *T. caecus* have inhabited Indian waters for approximately the last two decades and now both have well established populations. *T. caecus* specimens were collected in large quantities >100, indicating their potential for highly successful reproduction. Morphological and molecular analyses carried out in this research study, mark the first confirmed report of *T. caecus* in India.

Introduced species transfer is well known as a major vector for the worldwide movement of invasive species. Based on morphological features (shape of haptoral parts and the male copulatory organ), all monogeneans collected were described as *Thaparocleidus caecus*, and could not be separated morphologically from the original description by Mizelle and Kritsky 1969. The same species was

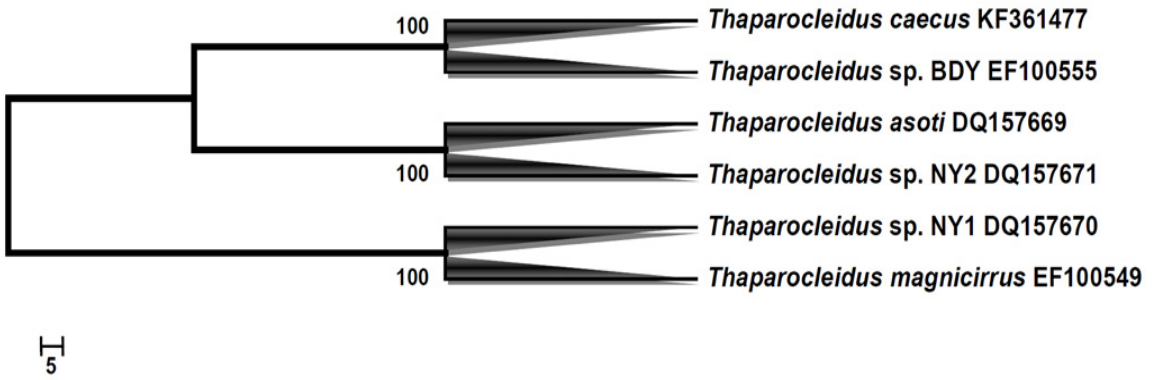


Figure 7. Maximum-parsimony (MP) tree for 28S region from selected *Thaparocleidus species* in GenBank (see accession numbers) including bootstrap values.

Table 1. Morphometrics of *Thaparocleidus caecus* infesting *Pangasianodon hypophthalmus*.

Characters	*Thaparocleidus caecus* Mizelle and Kritsky, 1969 (n= 20)	*Thaparocleidus caecus* Lim, 1990 (n= 10)	*Thaparocleidus caecus* Present specimen (n= 10)
Body Size:			
Length	904 (768-1055)	833 (333-1000)	580 (560-600)
Width	176 (140-251)	149 (124-183)	90 (80-100)
Haptor:			
Length	115 (96-137)	-	85 (80-90)
Width	144 (111-179)	-	70 (65-75)
Pharynx:	81 (68-95)	-	45 (42-48)
Male Copulatory Organ:			
Total length	66 (61-71)	62 (60-64)	65 (62-68)
Accessory piece	49 (40-54)	41 (40-44)	46 (44-48)
Dorsal Anchor:			
Total Length	46 (45-48)	-	52 (50-54)
Base Width	14 (12-15)	-	12 (10-14)
Inner Length	-	43 (40-44)	46 (44-48)
Inner Root	-	10 (8-12)	15 (14-16)
Recurved Point	-	12 (12-14)	18 (17-19)
Patch	-	8 (7-9) x 4(3-5)	8 (7-9) x 4(3-5)
Ventral Anchor:			
Total Length	21 (20-22)	20 (19-21)	24 (23-25)
Base Width	10 (9-11)	-	9 (8-10)
Recurved Point	-	8 (8-10)	9 (8-10)
Dorsal Bar:			
Length	32 (29-34)	44 (44-52)	35 (32-38)
Width	-	2 (2-4)	3 (2-4)
Ventral Bar:			
Total length	50 (44-59)	-	50 (44-58)
Length of one side	-	25 (22-26)	25 (22-28)
Hooks Length	12 (11-13)	11 (10-12)	11 (10-12)

also reported by Lim in 1990, from Malaysia, with slightly smaller sclerites. The BLASTN search in the NCBI (National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov) GenBank database revealed 98% match for the 28S rRNA with *Thaparocleidus* sp. BDY (EF100555). *Thaparocleidus* species was clustered together with *T. caecus* sequence in the maximum parsimony tree (Figure 8). Together with morphological identification and rRNA sequencing, *T. caecus* specimens are now confidently validated from Indian waters.

There is no information on the impact of *T. caecus* on local fish fauna in India. A high rate of infestation with monogenean parasites should be considered a threat for native fishes. Undetected parasites introduced by exotic fishes can affect endemic fishes more severely than their exotic carriers. In 1961, Dogiel noted that after the Caspian sturgeon was transferred to the Aral Sea, it introduced the gill monogenea *Nitzschia sturionis,* causing heavy mortalities in endemic sturgeon, the collapse of the sturgeon/caviar industry and almost exterminated the entire native sturgeon population. Introduction of non-native fish species to new geographical regions generally boosts fishery activities, but non-native fishes influence on the environment should be studied with risk awareness and caution given to the potential of parasitic transmission. Findings here show that this non-native monogenea is well established in the Meerut region of India, and now forms an exotic component of the Indian riverine system. This data and also new findings of *T. caecus* in other areas of India indicate the urgent need for proper monitoring programs in freshwater species introductions.

It is important to update records of Indian monogenean fauna. Further 28S sequencing is required from other *Thaparocleidus* species to reveal the clear position and validation of *T. caecus* within the Monogeneans. It is understood that once this parasitic flatworm is established, it is impossible to eradicate. Management strategies to further reduce the spread of this organism should include the study of vectors of invasion. Thus, a detailed monitoring of establishment, expansion and impact are urgently needed for proper management and control of non-native species. Efficient quarantine measures and checks for parasites should be introduced as important control measures for new species introductions. National policy and decision-makers in India should research the best way to legislate, in order to determine the safe import of non-native species.

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