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# Monogenoidea on exotic Indian freshwater fishes. 2. Range expansion of *Thaparocleidus caecus* and *T. siamensis* (Dactylogyridae) by introduction of striped catfish *Pangasianodon hypophthalmus* (Pangasiidae)

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**Summary**

*Thaparocleidus caecus* and *Thaparocleidus siamensis* are parasitic monogeneans found on the gills of striped catfish *Pangasianodon hypophthalmus* (Pangasiidae), a native species of Southeast Asia. We report *T. caecus* and *T. siamensis*, for the first time in India, from the gills of aquarium-kept *P. hypophthalmus* (prevalence 40 % and 80 % respectively). We also report *T. siamensis* from the gills of pond-cultured *P. hypophthalmus* (prevalence 100 %); no specimen of *T. caecus* was observed on pond-cultured *P. hypophthalmus* (prevalence 0 %). Morphometric data of the studied parasites did not differ significantly from previous descriptions of the two species recovered in other geographic locations. Similarly, no consequential variation was found when 28S rDNA of *T. siamensis* from the present study was compared with that of *T. siamensis* from Malaysia available on GenBank. The present investigation adds to growing cases of freshwater monogeneans that have been concomitantly introduced worldwide as a result of aquarium and aquaculture trade.

Keywords: Range expansion; Monogenoidea; *Thaparocleidus siamensis*; *Thaparocleidus caecus*; *Pangasianodon hypophthalmus*; India

## Introduction

The Striped catfish *Pangasianodon hypophthalmus* Sauvage, 1878 (Pangasiidae), a native of Mekong, Chao Phraya, and Maeklong basins of Southeast Asia (Roberts & Vidhayanon, 1991), is an excellent candidate for aquaculture and aquarium trade. The fish has been introduced as a source of aquaculture into river basins of many Asian countries (Roberts & Vidthayanon, 1991; Rainboth, 1996; Froese & Pauly, 2012), including India (Singh & Lakra, 2012). In addition, it is exported to 136 countries across all continents (Silva & Phuong, 2011), and its juveniles are available as ornamental fish for the aquarium trade in many countries (Baska *et al*., 2009). In fact, the farming of *P. hypophthalmus* has achieved such phenomenal growth over the last three decades that it has become a 'global icon of aquaculture development' (Silva & Phuong, 2011) with its production and distribution levels matching that of tilapia, carps and salmon. Despite this increasing socioeconomic importance of *P. hypophthalmus* culture, the parasitic fauna associated with this enterprise is not well studied (Thuy *et al*., 2010; Silva & Phuong, 2011).

## Material and methods

As part of our continuing effort on cataloguing the monogenean parasites (Platyhelminthes) from exotic Indian freshwater fishes (see Tripathi *et al*., 2010), we found specimens of *Thaparocleidus caecus* (Mizelle & Kritsky, 1969) Lim, 1996, and *Thaparocleidus siamensis* (Lim, 1990) Lim, 1996 from the gills of *P. hypophthalmus* purchased from the aquarium market of Lucknow (26° 50' N / 80° 56' E) (21.08.2012). Additionally, we found *T. siamensis* from the gills of *P. hypophthalmus* collected from the fish farms at Barabanki (26° 55' N / 81° 11' E) (24.07.2011). The prevalence and intensity of infestation of the worms isolated in the present study were recorded (Table 1 and 2). Some parasite specimens were mounted in glycerine and Canada balsam for studying their hard and soft body parts respectively, using the protocol recommended by Kritsky *et al*. (1986); others were preserved in absolute ethanol for DNA extraction. The mounted parasites were photographed with a digital camera (Olympus photometrics coolsnap) attached to a phase-contrast microscope (Olympus BX51). Based on these photographs, measurements were obtained with the software ProExpress 6.0 (Media Cybernetics, Inc., USA). Voucher specimens have been deposited in the Natural History Museum, London (2013.8.12.1-10, 2013.8.12.11-20).

Table 1. Prevalence and intensity of *T. caecus* and *T. siamensis* infestation on aquarium-kept *P. hypophthalmus*

| | *T. caecus* | *T. siamensis* |
|---|---|---|
| Prevalence | 40 % (4/10) | 80 % (8/10) |
| Intensity | 30 + | 200 + |

Table 2. Prevalence and intensity of *T. caecus* and *T. siamensis* infestation on pond-cultured *P. hypophthalmus*

| | *T. caecus* | *T. siamensis* |
|---|---|---|
| Prevalence | 0 % (0/4) | 100 % (4/4) |
| Intensity | 0 | 250 + |

DNA was extracted from the individual specimen of *T. siamensis* (query species) using the Qiagen's Dneasy blood and tissue Kit (Qiagen, Germany). The extracted DNA (10 µl) was used as a template in a PCR reaction to amplify the partial 28S rDNA, using forward (5'-ACCCGCTGAATTTAAGCAT-3') and reverse (5'-CTCTTCAGAGTACTTTTCAAC-3') primers. The reaction volume was 25 µl, containing 2 µl polymerase chain reaction (PCR) buffer (10X), 0.5 µl dNTPs (10 mM), 0.5 µl forward primer (19.6 nMol.), 0.5 µl reverse primer (31.9 nMol.), 0.5 µl Taq polymerase (5 Units), 1 µl MgCl2 (25 mM), 5 µl genomic DNA and 15 µl miliQ water. PCR conditions were 95 °C for 4 min (initial denaturation), followed by 35 cycles of 95 °C for 1 min (denaturation), 55 °C for 45 sec (annealing), 72 °C for 1 min (extension) and 72 °C for 10 min (final extension). An aliquote (10 µl) from the amplicon was electrophoresed in a 1.5 % agarose gels in TAE buffer, stained with ethidium bromide, and visualized under UV illuminator. The remaining amplicons were sequenced with the same primers using 3730/ABI-3730XL-1409-023 automated sequencer (Xcelris Labs Limited, India).

Sequencing products were subjected to BLAST (Basic Local Alignment Search Tool) for homology search. Aligned sequences of 28S rDNA of *T. siamensis* from the present study and that of Malaysia (available on GenBank under accession number AF218123.1) were compared using the SDSC (San Diego Supercomputer Center) work bench, and multiple sequence alignments were made by ClustalW (Thompson *et al*. 1994). Texshade (colour coded plots) of aligned sequences was used for conserved, non-conserved and identical sequences. Sequences were deposited in GeneBank under the accession number JX947852.

## Results and discussion

*Taxonomy*

*Thaparocleidus caecus* was originally described as *Ancylodiscoides caecus* Mizelle and Kritsky, 1969 from an unidentified aquarium fish collected in United States (Mizelle & Kritsky, 1969). Gussev (1978), however, transferred the species to *Silurodiscoides* Gussev, 1976 as *Silurodiscoides caecus* (Mizelle & Kritsky 1969) n. comb. Lim (1990) redescribed *S. caecus* based on new material and also described the new species, *S. siamensis*, both from *Pangasianodon hypophthalmus* pond cultured in Malaysia. Subsequently, Lim (1996) considered *Silurodiscoides* Gussev, 1976, a junior subjective synonym of *Thaparocleidus* Jain, 1952, based on the law of priority. Accordingly, the scientific names of *S. caecus* and *S. siamensis* were changed to *T. caecus* (Mizelle & Kritsky, 1969) Lim, 1996 and *T. siamensis* (Lim, 1990) Lim, 1996 respectively. We compared our specimens of *T. caecus* and *T. siamensis* from India with the respective redesrciption and description by Lim (1990), which we considered detailed and the most adequate representation of the two species. The general morphology and measurements of male and female reproductive organs, and haptoral parts of our specimens (Figs. 1 and 2) are consistent with the details provided by Lim (1990), and the diagnostic features of *Thaparocleidus*

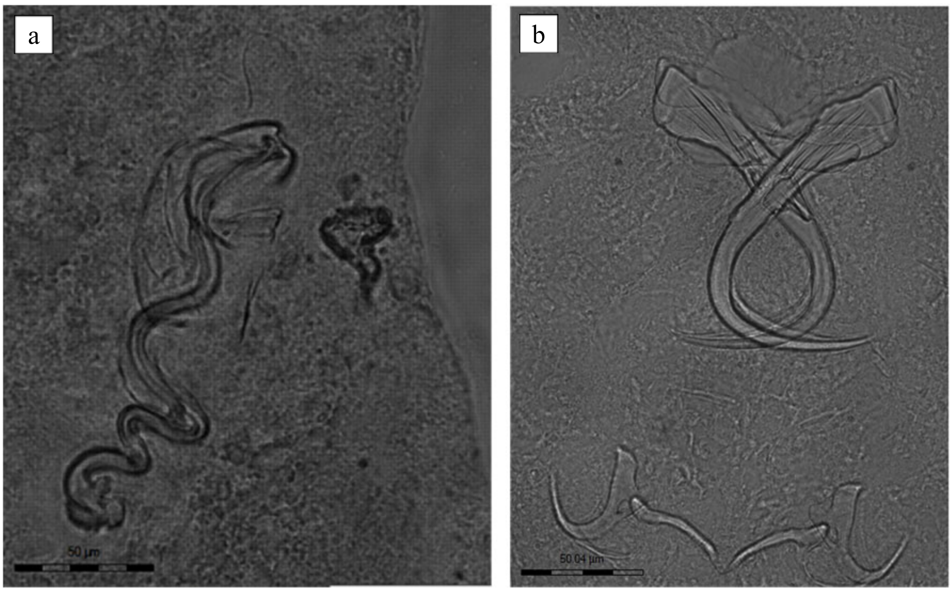


Fig. 1. Copulatory complex and vagina (a) and haptoral sclerites (b) of *Thaparocleidus siamensis* (Lim, 1990) Lim, 1996. Scale bar = 50 µm.

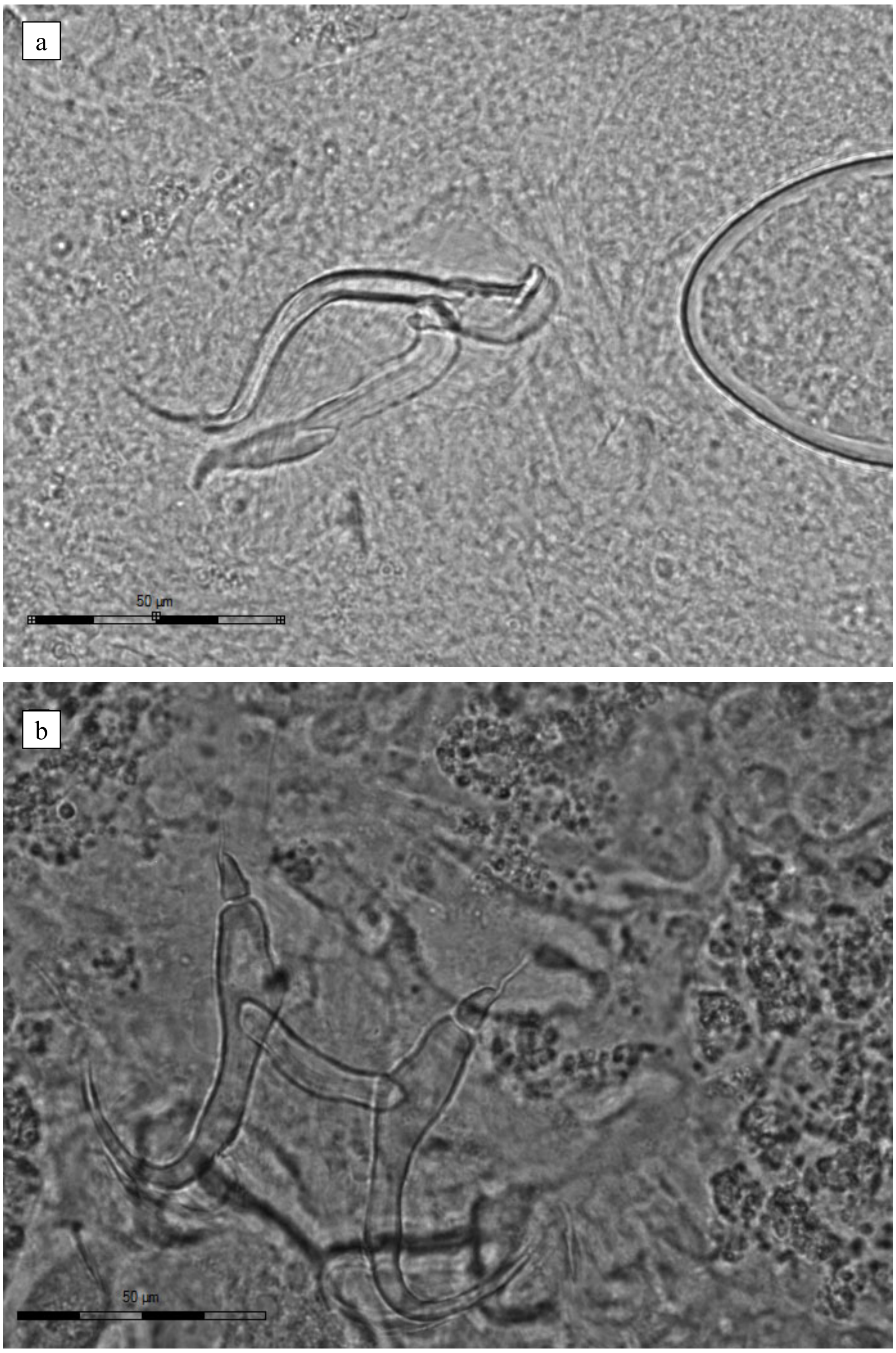


Fig. 2. Copulatory complex (a) and haptoral sclerites (b) of *Thaparocleidus caecus* (Mizelle & Kritsky, 1969) Lim, 1996. Scale bar = 50µm.

(Lim *et al*., 2001). Additionally, we have shown the morphology of the vagina to be inverted cone shaped in *T. siamensis* which Lim (1990) missed (Fig. 1). The morphometric data for *T. caecus* and *T. siamensis* examined are presented in Table 3 and Table 4 and compared with data reported by other authors. Molecular analysis also demonstrated that the specimens of *T. siamensis* from India, and those in Malaysia differ by only five bases (Figs. 3 and 4). In other words, the specimens of *T. siamensis* from India and those in Malaysia have 99.3 % similarity (MVIEW Multiple Alignment Display) and are thus similar species.

*Range expansion*

Mizelle and Kritsky (1969) described *T. caecus* from an unidentified aquarium fish imported into the United States from Thailand. Subsequently, Lim (1990) redescribed *T. caecus* from *P. hypophthalmus* (imported from Thailand and cultured) in Malaysia. Lim (1990) also described *T. siamensis* on the same host. Lerssutthichawal *et al*. (1999) recorded *T. caecus* from three host fishes: *Pangasius conchophilus*, *Pangasius larnaudii* and *P. hypophthalmus*, and *T. siamensis* from *P. hypophthalmus* cultured in Thailand. Pariselle *et al*. (2002) recorded *T. caecus* from both *P. hypophthalmus* and *Pangasius djambal* and *T. siamensis*

Table 3. Morphometric data (presented in µm as means followed by ranges in parentheses) of *Thaparocleidus caecus* from *Pangasianodon hypophthalmus* in India (present study), Malaysia (Lim 1990), and USA (Mizelle & Kritsky 1969)

| Parameters measured | Present study | Lim (1990) | Mizelle & Kritsky (1969) |
|---|---|---|---|
| **Body** | | | |
| Total body length | 1038 (680 – 1230) | 833 (333 – 1000) | 904 (768 – 1055) |
| Total body width | 167 (120 – 200) | 149 (124 – 183) | 176 (140 – 251) |
| Pharynx diameter | 48 (36 – 60) | – | 81 (68 – 95)* |
| Haptor length | 125 (80 – 150) | – | 115 (96 – 137) |
| Haptor width | 110 (77 – 150) | – | 144 (111 – 179) |
| **Male reproductive organs** | | | |
| Testis length | 113 (36 – 150) | – | – |
| Testis width | 63 (30 – 80) | – | – |
| Copulatory tube length | 60 (48 – 70) | 62 (60 – 64) | 66 (61 – 71) |
| Accessory piece length | 43 (34 – 53) | 41 (40 – 44) | 49 (40 – 54) |
| **Female reproductive organs** | | | |
| Ovary length | 141 (60 – 120) | – | – |
| Ovary width | 67 (40 – 100) | – | – |
| **Haptoral parts** | | | |
| Dorsal anchor length | 39 (37 – 41) | 43 (40 – 44) | 46 (45 – 48) |
| Dorsal anchor recurved point length | 11 (10 – 13) | 12 (12 – 14) | – |
| Dorsal anchor patch length | 6 (4 – 7) | 8 (7 – 9) | – |
| Ventral anchor length | 17 (16 – 19) | 20 (19 – 21) | 21 (20 – 22) |
| Ventral anchor recurved point length | 6 (5 – 7) | 8 (8 – 10) | – |
| Dorsal bar length | 42 (29 – 44) | 44 (44 – 52) | 32 (29 – 34) |
| Ventral bar length | 25 (21 – 27) | 25 (22 – 26) | 50 (44 – 59) |
| Hooks length | 14 (14 – 15) | 11 (10 – 12) | 12 (11 – 19) |
| **Egg** | | | |
| Egg length (with filament) | 77 (56 – 91) | – | – |
| Egg width | 41 (33 – 50) | – | – |

*Width of pharynx

from *P. hypophthalmus* (imported from Thailand and cultured) in Indonesia. They also described *T. vietnamensis* on *P. hypophthalmus* and *Pangasius bocourti*. Das *et al.* (2006) recorded *T. siamensis* from *P. hypophthalmus* (imported from Thailand and cultured) in Bangladesh. Thuy & Buchmann (2008) recorded both *T. siamensis* and *T. caecus* from *P. hypophthalmus* cultured in Vietnam. Wiecaszek *et al.* (2009) recorded *T. caecus* from an aquarium escapee pangasiid (either *P. hypophthalmus* or the hybrid of *P. hypophthalmus* with some unknown ornamental fish, presumably imported from Thailand) in Poland. Baska *et al.* (2009) recorded '*Thaparocleidus* monogeneans' from *P. hypophthalmus* fry (imported from Thailand to pet fish shops) in Hungary and Russia; although parasites could not be identified to species level, given the high level of monogenean host specificity, and their coevolution with their host, they were possibly either *T. siamensis* or *T. caecus* or even both.

In India, Rastogi *et al.* (2008) were apparently the first to record a monogenean parasite from aquarium-kept *Puntius sutchii* (now *Pangasianodon hypophthalmus*). However, these authors completely misidentified the parasite species for the formerly described *Silurodiscoides vistulensis* (Siwak 1932) Gusev 1973 (now *Thaparoclidus vistulensis* (Siwak 1932) Lim 1996). The copulatory organ, as illustrated by Rastogi *et al.* (2008), in particular, is not even close to that of *T. vistulensis*. Indeed, the monogenean illustrated by Rastogi *et al.* (2008) corresponds more to *T. siamensis* than *T. vistulensis*, to which this article is a testimony. Singh & Lakra (2012) also noted 'gill fluke infection' in all *P. hypophthalmus* farms in India, but neither the generic nor the specific identity of these parasites was established. Thus, ours is the first published account of *T. siamensis* and *T. caecus* from India, which also represents

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T._siamensis      ------------------------------------------------GAAAGAGATTAG
Query_sp.         GGCATGTGAGCAGAAACTGACCATGGCTTCTCTTAGTAACGGCGAGTGAAAAGAGATTAG
                                                                 ***********

T._siamensis      CCCATCACCGAAGCCCATCCGCTCATGTGGATAAGGCCATGTGGTGTTCAGTCATTGACC
Query_sp.         CCCATCACCGAAGCCCATCCGCTCATGTGGATAAGGCCATGTGGTGTTCAGTCATTGACC
                  ************************************************************

T._siamensis      TGGGGACAGTTGTTTACTCGAAGTCCAACTCCGATTTTGGCTTTGGATTTGTTCCGTAGA
Query_sp.         TGGGGACAGTTGTTTACTCGAAGTCCAACTCCGATTTTGGCTTTGGATTTGTTCCGTAGA
                  ************************************************************

T._siamensis      GGGTGAAAGGCCCGTACGARTAAACACTTACGGTATCAAGCTGTTTCCTAATGGTCAGTG
Query_sp.         GGGTGAAAGGCCCGTACGAGTAAACACTTACGGTATCAAGCTGTTTCCTAATGGTCAGTG
                  ******************* ****************************************

T._siamensis      ACATGGAGTCGGATTGCTTGAGAATGCAGTCCAAAGTGGGTGGTAAACTCCATCCAAGGC
Query_sp.         ACATGGAGTCGGATTGCTTGAGAATGCAGTCCAAAGTGGGTGGTAAACTCCATCCAAGGC
                  ************************************************************

T._siamensis      TAGATACTGGCACGAGTCTCG---------------------------------------
Query_sp.         TAAATACTGGCACGAGTCCGATAGTAGACAAGTACCGCGAGGGAAAGTTGAAAAGTACTC
                  ** ***************

T._siamensis      -------------------------------
Query_sp.         TGAAGAGAGAGTAAATAGTACGTGGATACCC
```

Fig. 3. ClustalW alignment

a range extension of these two species to South Asia. The extent of global distribution of these two parasite species also exemplifies the potential of exotic fish to concomitantly introduce their monogeneans into areas outside of their natural range (see Tripathi, 2013 for discussion).

*Disease risk implications*

The pathological implications of these monogeneans are not well studied. Nonetheless, the sporadic mortality of *P.*

Table 4. Morphometric data (presented in µm as means followed by ranges in parentheses) of *Thaparocleidus siamensis* from *Pangasianodon hypophthalmus* in India (present study) and Malaysia (Lim 1990)

| Parameters measured | Present study | Lim (1990) |
|---|---|---|
| **Body** | | |
| Total body length | 822 (695 – 1139) | – |
| Total body width | 140 (90 – 170) | – |
| Pharynx diameter | 40 (30 – 50) | – |
| Haptor length | 118 (90 – 175)) | – |
| Haptor width | 93 (70 – 145) | – |
| **Male reproductive organs** | | |
| Testis length | 131 (90 – 180) | – |
| Testis width | 64 (45 – 95) | – |
| Copulatory tube length | 96 (90 – 97) | 97 (90 – 100) |
| Accessory piece length | 129 (121 – 138) | 120 (110 – 134) |
| **Female reproductive organs** | | |
| Ovary length | 124 (90 – 188) | – |
| Ovary width | 65 (50 – 85) | – |
| Vagina length | 37 (20 – 50) | – |
| **Haptoral parts** | | |
| Dorsal anchor length | 60 (58 – 64) | 64 (60 – 70) |
| Dorsal anchor recurved point length | 30 (27 – 35) | 33 (28 – 38) |
| Dorsal anchor patch length | 30 (24 – 35) | 30 (24 – 36) |
| Ventral anchor length | 19 (15 – 21) | 23 (22 – 24) |
| Ventral anchor recurved point length | 12 (9 – 15) | 13 (12 – 14) |
| Dorsal bar length | 38 (31 – 40) | 40 (36 – 44) |
| Ventral bar length | 28 (25 – 32) | 28 (24 – 30) |
| Hooks length | 12 (12 – 13) | 14 (13 – 15) |
| **Egg** | | |
| Egg length (with filament) | 237 (147 – 385) | – |
| Egg width | 25 (19 – 30) | – |

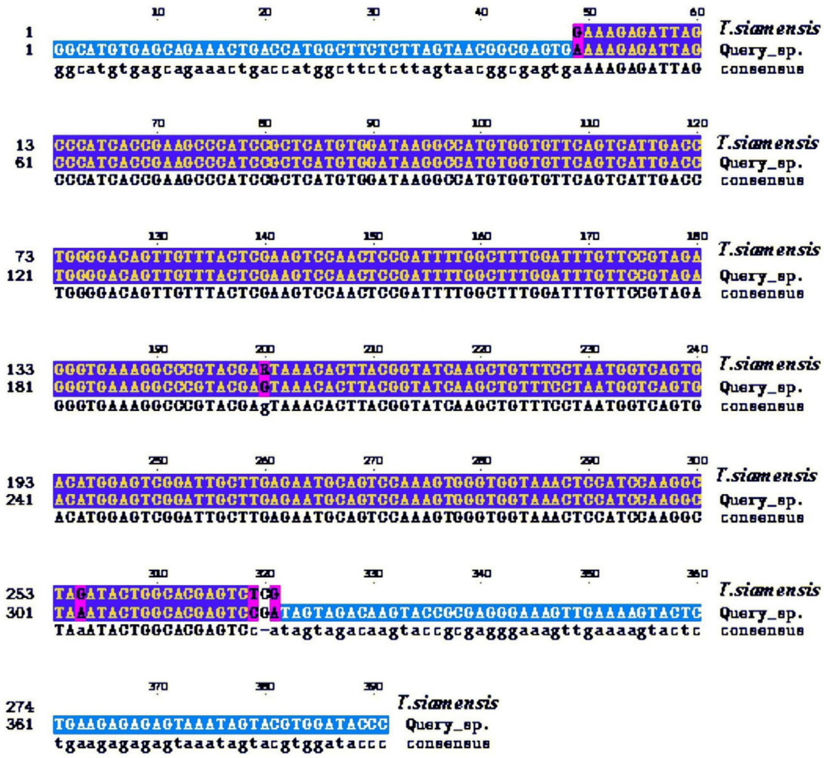


Fig. 4. Texshade showing colour coded plots for conserved sequence (light blue colour), all match sequence (blue colour) and similar sequence (purple colour) and non-match sequence (white colour)

*hypophthalmus* due to *T. siamensis* and *T. caecus* infestation has been documented in Vietnam (Thuy & Buchmann, 2008). In India, Singh and Lakra (2012), who were not aware of taxonomic status of monogeneans from *P. hypophthalmus*, also reported, "gill fluke infection common in all *P. hypophthalmus* farms with infection rate varying from 60 – 90 % and highest mortality during the first week after stocking". Indeed, the intensities of infestation by *T. siamensis* in the present study reached higher than 200 parasites per aquarium-kept fish and 250 parasites per pond-cultured fish, which makes it difficult to envisage that such a high parasitic burden would not exert a strongly adverse effect on the host survival. Further studies are required to estimate their pathogenicity accurately.

An additional disease risk involved, especially in Indian conditions where the culture sites of *P. hypophthalmus* are very close to open water (Singh & Lakra, 2012), is the spread and colonisation of *P. hypophthalmus* and associated monogeneans to sympatric wild fish. The fish was previously known to have established a small population of it in the lake Kinneret, Israel as an aquarium escapee (Snovsky & Golani, 2012) and is thus capable of establishing elsewhere, including India. Should the escapee *P. hypophthalmus* establish its population in Indian waters, it can trickle across state boundaries and disseminate its monogeneans to further new localities for a combination of two reasons: 1) *P. hypophthalmus* is highly potamodromous and can cover distances of over several hundred kilometres (FAO 2013), and 2) India has one of the largest networks of rivers in the world (Rao, 1975).

Wiecaszek *et al*. (2009) contended that *T. caecus*, because of its very narrow host-specificity, poses no threat to the native ichthyofauna of Europe. However, a thorough examination of the literature reveals that *T. caecus* can actually exploit a range of pangasiid hosts (Table 3). Evidently, *T. caecus* is a generalist parasite with wide host specificity, and thus most likely to represent a natural threat to native fish biodiversity of importing countries.

Table 5. Fish species reported as hosts of *Thaparocleidus caecus*

| **Host fish** | **Reference** |
|---|---|
| **Pangasiidae** | |
| *Pangasianodon hypophthalmus* | Lim (1990); Lerssutthichawal *et al*. (1999); Pariselle *et al*. (2002); Thuy & Buchmann (2008); Wiecaszek *et al*. (2009); Baska *et al*. (2009); Present study |
| *Pangasius conchophilus* | Lerssutthichawal *et al*. (1999) |
| *Pangasius larnaudii* | Lerssutthichawal *et al*. (1999) |
| *Pangasius djambal* | Pariselle *et al*. (2002) |

Considering continuing worldwide introductions/exports of *P. hypophthalmus* for aquarium and/or aquaculture purposes, we anticipate further range expansion for these parasites. It seems only relevant and essential that parasitologists consider the potential impact of parasites onto the economically important *P. hypophthalmus* farming.

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