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Short Communication

A Note on *Thaparocleidus caecus* (Mizelle & Kritsky, 1969) (Monogenea: Dactylogyridae) Detected with Morphological and Molecular Tools in *Pangasianodon hypophthalmus* (Sauvage, 1878) Imported into Turkiye

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ABSTRACT

Thaparocleidus caecus (Mizelle & Kritsky, 1969) Lim, 1996 (Dactylogyridae) was reported for the first time from iridescent shark-catfish (*Pangasianodon hypophthalmus*, Sauvage, 1878) imported into Turkiye via the aquarium fish trade. *T. caecus* were found on the gills of the examined fish with a prevalence of 28%. Identification, based on morphological characteristics for *T. caecus*, was confirmed by rRNA sequencing, evidencing the transfer of *T. caecus* to different countries. Our results emphasize that the ornamental fish trade poses a range expansion of un-documented parasite species.

Keywords: *Thaparocleidus caecus*, *Pangasianodon hypophthalmus*, shark-catfish, parasite transfer, aquarium fish trade

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INTRODUCTION

Aquaculture and the ornamental aquatic animal trade provide access to the dispersal of non-native aquatic animal species (Peeler et al., 2011). The economic benefits of non-native introductions as in the tropical aquarium fish trade are clear. However, the movement of live animals is a serious risk for the spreading of diseases (Evans & Lester, 2001). It is known that the main locations of ornamental fish production are in Southeast Asian countries (Kim, Hayward, & Heo, 2002). Turkiye also imports various tropical aquarium fish from these countries; the imported fish species are wide in scale and number. The possibility of disease transmission via the main route of the live fish trade was reported in previous studies (Evans & Lester, 2001; Kim, Hayward, & Heo, 2002; Yildiz, 2005; Gjurčević et al., 2007; Kayis et al., 2009; Koyuncu, 2009; Tavares-Dias, Lemos, & Martins, 2010; Peeler et al., 2011; Trujillo-González et al., 2018; Trujillo-González et al., 2019). In these studies, different parasites recovered from the aquarium fish have commonly been identified: *Dactylogyrus* sp., *Gyrodactylus* sp., *Argulus* sp., *Ichthyobodo* sp., *Ichthyophthirius multifiliis* (Fouquet, 1876), *Trichodina reticulate* (Hirschmann & Partsch, 1955), *Capillaria* sp. and *Lernaea cyprinacea* (Linnaeus, 1758). There is no record of the occurence of *Thaparocleidus caecus* (Mizelle & Kritsky, 1969) Lim, 1996 in the imported *Pangasianodon hypophthalmus* (Sauvage, 1878) to Turkiye. However, the *Thaparocleidus* species; *T. siluri* (Zandt, 1924) Lim, 1996 and *T. vistulensis* (Siwak, 1932) Lim, 1996 were previously reported in *Silurus glanis* (Linnaeus, 1758) in Lake Gala (Edirne, Turkiye) by Soylu (2014) and *T. caecus* in *Pangasius pangasius* (Hamilton, 1822) by Tokşen, Zoral, & Şirin (2014). Herein we report the transfer of *T. caecus* isolated from *Pangasianodon hypophthalmus* (Pangasiidae) fish imported into Turkiye together with a brief morphological description and molecular identification.

MATERIALS AND METHODS

Iridescent shark-catfish (*Pangasianodon hypophthalmus*) imported through Singapore were obtained from commercial suppliers within two working days of importation. The examined fish were clinically healthy with no signs of disease and selected randomly. The fish were examined with the permission of the Ankara University, Animal Ethical Committee (No:2015-12-136). The Fish were anaesthetically overdosed with clove oil, then humanely killed by severing the spinal cord with a sharp blade, and a complete ecto-parasitological examination was undertaken. A total of 54 shark-catfish (*P. hypophthalmus)* were examined under a microscope (Pritchard & Günther, 1982). The monogenean parasites found during examination were fixed in 70% alcohol and mounted in glycerin. The mounted parasites were photographed with a trinocular microscope (Leica CME) and the morphometric data of the parasite were obtained by Micro-cam version 5.5. The basic morphological keys used in the species identification were the sclerotized part of the attachment as well as the morphology and the length of the male reproductive organ in the parasite (Mizelle & Kritsky, 1969; Lim, 1990; Lim, 1996; Pariselle, Lim, & Lambert, 2006).

For molecular analysis, monogeneans were preserved in absolute alcohol. The prevalence (%) and the mean intensity of the parasite were assessed as reported by Bush et al. (1997). For molecular discrimination, a Qiagen D Neasy Blood & Tissue Kit was used for DNA extractions. The quantity and quality of the extracted DNA was assessed with a Colibri nano-spectrophotometer. The template DNA was adjusted to a final concentration of 50 ng/µL. The amplification of 649-bp-long fragment from the 5′ region of the partial 18S and ITS-1 region was done using a primer pair S1 (5′ ATTCCGATAACGAACGAGACT 3′) that binds with the terminal region of the 18S gene and H7 (5′ GCTGCGTTCTTCATCGATACTCG-3′) (Sinnappah et al., 2001). PCR amplifications were performed as previously described by Keskin, Unal, & Atar (2016). Briefly, 8 µl of 5x FIREPol Master Mix Ready to Load (Solis BioDyne, Estonia), 1 µl of each primer (F, R), template DNA (2 µl) and distilled water (28 µl) in a total reaction volume of 40 µl were used for the amplifications. The thermal cycler was run according to Keskin, Unal, & Atar (2016). An optimal fragment size of PCR products was checked using agarose gel (2%) electrophoresis. QIAGEN QIAquick Gel Extraction kit was used for Purification of PCR products. Sequencing was performed on an ABI Prism 310 genetic analyzer, using both primers for bidirectional sequencing. The ClustalW was used for the alignment of nucleotide sequences (Thompson et al., 1994) and edited using MEGA 6 (Tamura et al., 2013). Taxon DNA/Species Identifier v1.7.7 was applied to quantify the proportion of correctly identified queries according to Best Close Match (BCM), with a 3.0% threshold (Meier et al., 2006). The barcode sequence was trimmed by 649 base pairs with a reference sequence FJ493153.

RESULTS AND DISCUSSION

The ectoparasite found on the gills of *P. hypophthalmus* was identified as *Thaparocleidus caecus* (Figure 1).

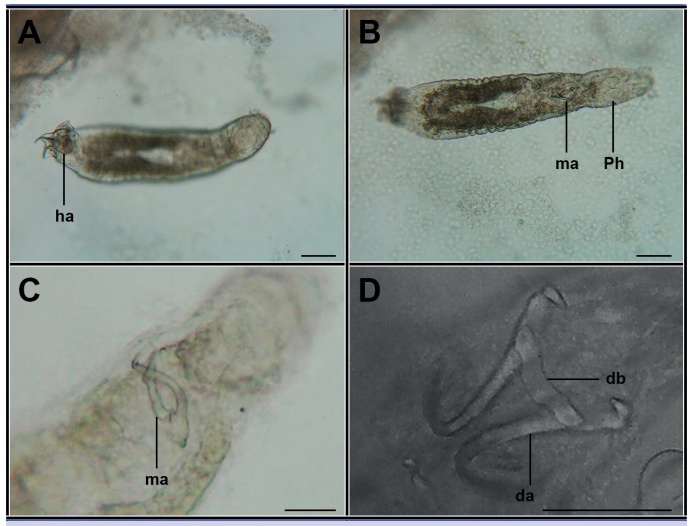

Figure 1. *Thaparocleidus caecus* recovered from the gills of *Pangasianodon hypophthalmus* A) ha: haptoral armature B) ma: male apparatus, ph: pharynx C) ma: male apparatus D) da: dorsal anchor, db: dorsal bar (scale bar = A,B:100 µm; C,D: 50 µm).

Of the quantitative parasite descriptors of *T. caecus* in *P. hypophthalmus*, the prevalence was found to be 28% and mean intensity 37.73± 10.15 (Table 1).

Table 1. The prevalence and mean intensity of *T. caecus* recovered from the gills of *P. hypophthalmus.*

Host fish species	Parasite species	Examined Fish Number	Prevalence (%)	Mean Intensity (MI±SE)
P. hypophthalmus	*T. caecus*	54	28	37.73±10.15

(MI: mean intensity, SE: standart error)

T. caecus (Dactylogyridae) described in the gill filaments of freshwater iridescent shark-catfish, *P. hypophthalmus* has been imported into Turkiye from Asia. The literature exists for the parasite species described here and its host. Monogeneans from Pangasiidae with their morphological and molecular characteristics were previously reported by Lim, Timofeeva, & Gibson (2001), Pariselle, Lim, & Lambert (2006), Thuy & Buchmann (2008), Šimková, et al. (2013), Chaudhary et al. (2014), Tripathi, Rajvanshi, & Agrawal (2014). The *Thaparocleidus* species are gill monogeneans showing host specific character for the Siluriform group of fish (Lim, 1996).

The *Thaparocleidus* species found in different Pangasiidae (Siluriformes) were described in detail by Pariselle, Lim, & Lambert (2005) and Pariselle, Lim, & Lambert (2006). The prevalence of *T. caecus* found in *P. hypophthalmus* showed similarity with the record of prevalence for *T. caecus* exists in the same fish in India (Tripathi, Rajvanshi, & Agrawal, 2014).

The morphological characteristics of *T. caecus* were four granulated eyespots, an elongated body, a circular pharynx, a male capulatory organ, a dorsal anchor larger than the ventral anchor, 14 marginal hooklets and a v-shape ventral bar (Figure 1). The total body length and width of *T. caecus* were measured to be 772.55±31.13 µm and 153.88±9.87 µm, respectively. The morphometric data on the parasite are presented in Table 2.

Table 2. Morphometric data of *Thaparocleidus caecus* in *Pangasianodon hypophthalmus*.

Characters measured (µm)	(Mean±SE) (Min-Max)
Body	
Total body length	772.55±31.13 (614,11-992,28)
Total body width	153.88±9.87 (60,82-197,71)
Pharynx diameter	74.17±2.65 (71-79)
Male apparatus	
Male capulatory organ lenght	64.19±3.13 (58-71.25)
Male accessory piece	46.39±3.46 (40-56)
Dorsal anchor	
Dorsal anchor length	54.53±4.87 (47-64)
Dorsal anchor width	11.35±0.38 (11-12)
Dorsal bar length	46.30±1.56 (44.74-47.86)
Ventral anchor	
Ventral anchor length	28.88±0.42 (28-29)
Ventral bar length of one side	29.07±1.42 (28-30)

Morphometric characters measured are given in mean (µm) ±SE (standard error) followed by minimum-maximum (Min-Max) values in parentheses.

The morphological structure and morphometric data of the male copulatory organ and attachment organ are considered important criteria in the species identification of *Thaparocleidus* (Šimková, et al., 2013). In our study, the measured data of attachment parts including anchors (ventral and dorsal) and the male copulatory organ size were within the ranges of previous reports on *T. caecus* by Mizelle & Kritsky (1969), Lim (1990), Tripathi, Rajvanshi, & Agrawal (2014) and Chaudhary et al. (2014). The morphology of *T. caecus* assessed in this study also complies with these previous studies.

In the genetic analysis, the Basic Local Alignment Search Tool (BLAST) showed a 99% sequence similarity for species identification of *T. caecus*. A nucleotide composition analysis indicated 46.7% QC. The nucleotide sequence of *T. caecus* identified in this study was recorded in GenBank under the accession number MK464252. All of the confirmed positive *T. caecus* amplicons were 99% homologous to *T. caecus ITS1* GenBank sequences (FJ493153). Thus, the genetic analysis of *T. caecus* was previously documented by Šimková, et al. (2013), Chaudhary et al. (2014) and Tripathi, Rajvanshi, & Agrawal (2014).

Our results concluded that the parasites are continuously being transferred between various locations in the World with the route of the aquarium fish trade. The pathogens spreading to new areas with the host's movement is undoubtedly recognised. Ultimately, it is not a new problem but it does seek new solutions. Evidentially, *T. caecus* in *P. hypophthalmus* is being reported to be transferred to Turkiye for the first time with this study. Więcaszek et al. (2009) stated that monogenoid parasites found on Pangasiids imported from South-Asia to Europe have no potential threat due to their narrow host specificity. However, Tripathi (2014) highlighted the invasive potential of parasitic monogenoids including the *Thaparocleidus* species via the aquarium fish trade.

CONCLUSION

The detection of *T. caecus* in *Pangasianodon hypophthalmus* imported into Turkiye demonstrated that the parasite transfer within the ornamental fish trade is a challenging issue in relation to parasite dispersal between countries. Diversification of *Thaparocleidus*, associated with a host changeover, is a potential risk for the native fish of Turkiye. Considering the possible dangers of parasite transfer to native host populations and aquaculture, much more emphasis should be given on studies to assess the actual impact of the parasites on different fish species. The ornamental fish trade is a serious threat for parasite movement thus, a new strategy for "pre-border controls" to prevent parasites spreading via the aquarium fish trade should be developed.

Conflict of interests: Authors declare that there is no conflict of interest regarding the publication of this paper.

Ethics committee approval: The study protocol for fish was approved by Ankara University, Animal Ethical Committee (No:2015-12-136). Animals (fish) were handled according to the international animal ethical rules.

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