

Tri-hooked Monogenean Parasite (*Heteronchocleidus buschkieli*; Ancyrocephalidae: Heteronchocleidinae) Isolated from Ornamental Fish (*Colisa lalia*) Imported into Iran

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Makale Kodu (Article Code): KVFD-2013-10552

Summary

Parasites imported with dwarf gourami fish may present risks, both to native fish populations and to the aquaculture industry. *Heteronchocleidus buschkieli* Bychowsky, 1957 is an important Anabantidae fishes parasite. A large number of imported anabantid fish originate from Southeast Asian countries. The objective of this study was to investigate the occurrence of infection with monogenean parasite in dwarf gourami fish imported from Southeast Asia into Iran. In this study, a total of 400 dwarf gouramies were randomly selected from aquaria containing this fish imported from Southeast Asia into Iran. After euthanizing, the gills were removed and arches and skin were examined microscopically for monogenean parasites. *Heteronchocleidus buschkieli* (Monogenea: Ancyrocephalidae) was the only monogenean parasite identified morphologically in 120 of 400 (30%) imported fish. The infected fish were lethargic and had hyperemic gills with small skin lesions. Prevalence, abundance and mean intensity of infection for four years were 0.3, 30 and 30 ± 1.96 , respectively. To the author's knowledge, this is the first report of dwarf gourami infection with *H. buschkieli* in Iran. In order to prevent the introduction of this parasitic pathogen from imported fishes, we recommend imported fishes should be examined and treated before domestic distribution and quarantine practices should be implemented.

Keywords: Dwarf gourami, Ornamental fish, *Heteronchocleidus buschkieli*, Monogenea, Iran

İran'a Yurt Dışından Getirilen Süs Balıklarından (*Colisa lalia*) İzole Edilen Üç-Kancalı Monogenean Parazit (*Heteronchocleidus buschkieli*; Ancyrocephalidae: Heteronchocleidinae)

Özet

Cüce gurami balık ile taşınan parazitler hem endemik balık popülasyonlarına hem de akuakültür endüstrisine risk oluşturabilirler. *Heteronchocleidus buschkieli* Bychowsky, 1957 önemli bir Anabantidae balık parazitidir. Çok sayıda yurt dışından taşınan anabantid balık Güneybatı Asya ülkelerinden köken alır. Bu çalışmanın amacı İran'a Güneybatı Asya'dan ithal edilen cüce gurami balıkta monogenean parazit enfeksiyonunun mevcudiyetini araştırmaktır. Bu çalışmada, İran'a Güneybatı Asya'dan ithal edilen balıkları içeren akvaryumdan rastgele toplam 400 adet cüce gurami seçildi. Ötenaziye takiben solungaçlar uzaklaştırıldı ve yüzgeçler ile deri monogenean parazit için mikroskopta incelendi. *Heteronchocleidus buschkieli* (Monogenea: Ancyrocephalidae) ithal edilen balıklardan 400'ünden 120'sinde tespit edilen tek monogenean parazitti. Enfekte balıklar halsiz olup hiperemik solungaçlara ve küçük deri lezyonlarına sahipti. Dört yıl için enfeksiyonun prevalans, çokluk ve ortalama yoğunluğu sırasıyla 0,3, 30 ve $30 \pm 1,96$ idi. Yazarların bilgisi kapsamında bu çalışma İran'da *H. buschkieli* ile enfekte cüce guraminin varlığını ortaya koyan ilk rapordur. İthal edilen balıklardan bu parazitik patojenin oluşumunu engellemek amacıyla bizler ithal edilen balıkların yurt içine yayılmadan ve karantina uygulanmadan önce bu balıkların kontrol ve tedavi edilmeleri gerektiğini tavsiye ederiz.

Anahtar sözcükler: Cüce gurami, Süs balığı, *Heteronchocleidus buschkieli*, Monogenea, İran

INTRODUCTION

Until the second half of the 20th century, the Aspidogastrea, Digenea and Monogenea (Monogenoidea) were considered together as 'trematodes'. The Monogenea

was converted to class level by Bychowsky ^[1]. Class monogenoidea is divided into 2 subclasses: 1) lower or Polyonchoinea, with 6 orders and about 30 families



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(e.g. Dactylogyridae, Gyrodactylidae, Capsalidae and Ancyrocephalidae) and 2) higher or Oligonchoinea with 5 orders and about 35 families (e.g. Discocotylidae, Mazocraeidae and Diplozoidae) [2,3].

Monogenea are microscopic to medium-sized (0.15 to 20.0 mm, rarely up to 30.0 mm) parasitic flatworm of fish generally in freshwater rivers and lakes. They have a direct life cycle and are either viviparous (e.g., Gyrodactylidae) or oviparous (e.g., Dactylogyridae) [3-6]. Monogeneans are host specific and infect only one or very few host species [2,7,8]. Oviparous species of Monogeneans cannot survive more than 2 weeks off a host (except their over-wintering eggs) [9].

Most monogeneans are ectoparasitic on specific sites of fish such as branchial arches, the fins, and the head. They attach to the host using their opisthaptor (an organ located at the posterior end of the monogen) which is usually equipped with 2 to 4 larger hooks, 12 nearly always to 16 marginal hooks and/or clamps or suckers [3-5]. The number and shape of these structures are important in monogenean classification [2,3]. Numerous species of monogeneans are known to cause morbidity and mortality in freshwater and marine fishes because of their high intensities, and significant damage at the point of attachment through their opisthaptor and by grazing on exposed organs and tissues [4]. Monogenean trematodes are serious pests of ornamental fishes particularly dactylogyrids causing damage to gill filament of fishes [5].

Heteronchocleidus Bychowsky, 1957 an Anabantidae fish parasite is a representative of infrequent tropical monogenetic trematodes related to Ancyrocephalidae (Ancyrocephalinae) [10]. The adult worms have three powerfully developed middle hooks, the fourth remains undeveloped [10,11]. The vestigial dorsal anchor (fourth hook) in all species of *Trianchoratus*, *Heteronchocleidus* and *Eutrianchoratus* those assigned to the subfamily Heteronchocleidinae Price, 1968 (previously regarding to Ancyrocephalidae Bychowsky, 1937) is comma-shaped [12]. Paperna remarked that there is an evolutionary circumstance beginning from two pairs of anchors to three anchors, by loss of one anchor [13]. Structural differences in anchors and bars are used to differentiate species, while the copulatory organ remains homologous in almost all fish species [11].

A significant portion of worldwide aquatic animals' trade consists of tropical aquarium fish such as anabantid fishes. The anabantid fishes vary in color and are found in most aquaria. They may use atmospheric air in order to breathe and an accessory breathing organ called the labyrinth organ (composed of lamella which are covered over a specific blood-soaked thin skin) is located next to the gill cavities [10,14,15]. A large number of imported anabantid fish originate from Southeast Asian countries.

Currently, there is limited information available regarding the occurrence of parasitic pathogens in imported dwarf

gourami (*Colisa lalia*, Hamilton 1822) into Iran. The objective of this study was to investigate the occurrence of infection with monogenean parasite in dwarf gourami fish imported from Southeast Asia into Iran.

MATERIAL and METHODS

From July 2008 to September 2012, a total of 400 dwarf gouramies (Anabantid fish) were randomly selected from aquaria containing dwarf gouramies imported from Southeast Asia into Iran. They were held in aquaria at 27±2°C. The fish were immediately transported to the Aquatic Laboratory, Faculty of Veterinary Medicine, Islamic Azad University. The animals were euthanized by decapitation following clove flower extract bathing. The monogenean parasites were defined and counted in fish gills and skin. The gills were removed and arches were placed one by one on separate slides. The slides were examined by light microscopy with 10 × magnification using a Laboval 4 microscope (Zeiss, Munich, Germany). The monogenean parasites were isolated and moved to another slide and were fixed and cleared by adding ammonium picrate [16]. Then, a cover slip was placed and sealed by paraffin. Finally, light microscopy at 40 × magnification was used for identification of the monogenean parasites. Using the same procedure, skin wet mount was also examined for the monogenean parasites.

Identification of monogenean species was done using morphological characteristics (shape and number of hooks and bars) according to Bychowsky [10] and Lim [17,18] identification keys. Briefly, the monogenean with three well developed anchors and one rudimentary anchor with two bars were confirmed as the genus *Heteronchocleidus*.

Key to the genera of *Heteronchocleidinae* Price, 1968 [18]

Monogeneans with three well developed anchors and one rudimentary anchor

- With 2 bars..... *Heteronchocleidus*
- With 1 bar..... *Eutrianchoratus*
- Without bar *Trianchoratus*

All statistical analyses were performed using GraphPad software (GraphPad software, Inc., La Jolla, CA, USA) and data were expressed as mean ± standard deviation (SD).

RESULTS

Heteronchocleidus buschkieli (Monogenea: Ancyrocephalidae) (Fig. 1) as the only monogenean parasites was identified with its specific three hooks and two bars (Fig. 2) in 120 of 400 (30%) imported fish. The infected fish were lethargic and small skin lesions (Fig. 3).

Overall, 7203 parasites were isolated from all 120 fish with a range of 48 to 73 parasites per each fish. In general,

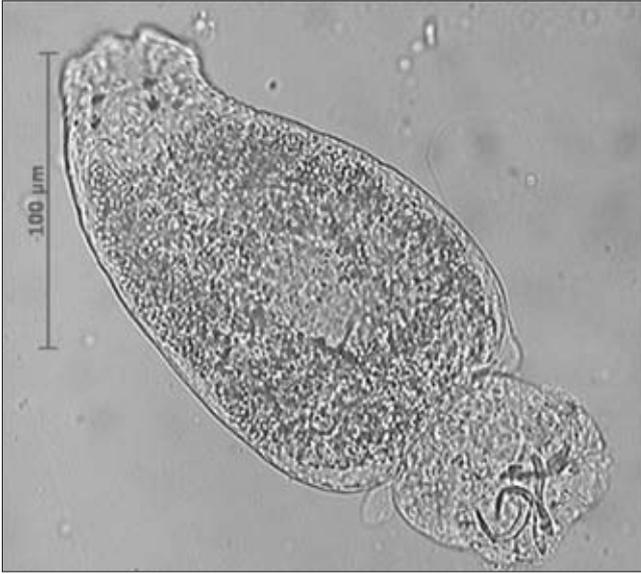


Fig 1. *Heteroncholeiidus buschkieli* isolated from ornamental fish (*Colisa lalia*)
Şekil 1. Süs balığından (*Colisa lalia*) izole edilen *Heteroncholeiidus buschkieli*

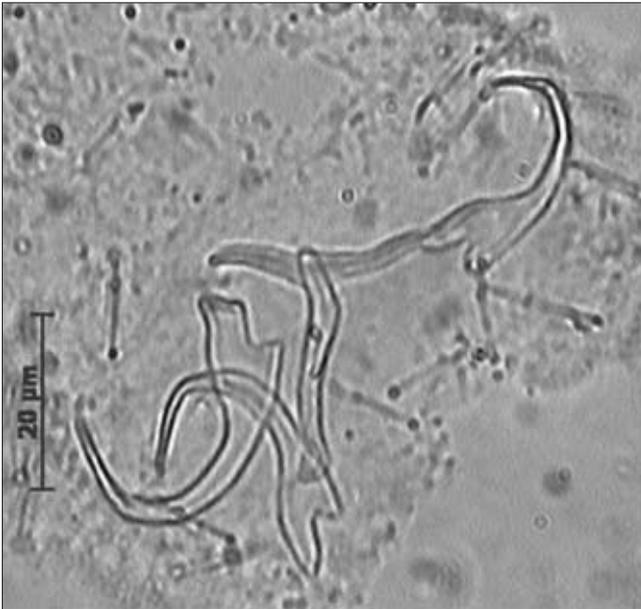


Fig 2. Three hooks and two bars of *Heteroncholeiidus buschkieli* parasite isolated from ornamental fish (*Colisa lalia*)
Şekil 2. Süs balığından (*Colisa lalia*) izole edilen *Heteroncholeiidus buschkieli* parazitinde üç kanca ve iki bar

5 to 9 parasites were counted in each infected gill arch and 2 to 7 parasites were counted in the skin wet mount. Mean quantity of this parasite in each fish was 60.03 ± 7.36 . With 95% Confidence Interval (CI), number of parasites in each fish was between 57.67 to 62.38. However, in the high-infected gill arches up to 25 parasites were detected per high ($\times 40$ objective) power field.

Prevalence, abundance, mean intensity and percentage of infection for four years are shown in [Table 1](#).



Fig 3. Dwarf gourami fish (*Colisa lalia*) with skin lesions
Şekil 3. Deri lezyonlu cüce gurami balığı (*Colisa lalia*)

Table 1. Prevalence, abundance, mean intensity and percentage of infected fish

Year	Prevalence	Abundance	Mean Intensity	Percentage of Infection
1 st year	0.47	47	47.04±3.45	47%
2 nd year	0.25	25	24.96±1.56	25%
3 rd year	0.21	21	21±1.29	21%
4 th year	0.27	27	27±1.54	27%
Mean for 4 years	0.3	30	30±1.96	30%

Table 2. Prevalence, abundance, mean intensity and percentage of parasitized fish

Year	Prevalence	Abundance	Mean Intensity	Percentage of Parasite
1 st year	0.0084	61.04	61.04±6.87	0.84 %
2 nd year	0.0082	59.09	59.09±7.64	0.82%
3 rd year	0.0081	58.78	58.78±7.95	0.81%
4 th year	0.0085	61.22	61.22±6.98	0.85%
Mean for 4 years	0.0083	60.03	60.03±7.36	0.83%

Prevalence, abundance, mean intensity and percentage of parasites for four years are shown in [Table 2](#).

DISCUSSION

In the present study, the dwarf gourami imported from Southeast Asia presented a great degree of infection with *H. buschkieli*. To the author's knowledge, this is the first report of dwarf gourami infection with *H. buschkieli* in Iran. Infection with this parasite in gills of small aquarium fish *Macropodus opercularis* (paradise fish) was first reported by Bychowsky [10]. Currently, there are 29 recognized

species of heteronchocleidine monogeneans including *Trianchoratus* spp. (10 species), *Eutrianchoratus* spp. (9 species), and *Heteronchocleidus* spp. (10 species) isolated from freshwater fish species of channids (3 species) and anabantoids (13 species)^[12]. But its host specificity limits to anabantid freshwater fishes.

H. buschkieli from croaking gourami (*Trichopsis vittatus*) has been found in the gills of 7 species of freshwater fishes in Malaya^[17]. Chang and Ji have reported infection with *H. buschkieli* and *H. magnihamatus* in gills of *Macropodus chinensis* in China^[19]. Recently, monogeneans such as *H. buschkieli*, *H. gracilis* Mizelle and Kritsky, 1969 and *H. colisai* Chandra and Yasmin, 2003 have been reported from India^[11].

Heteronchocleidus sp. has been observed in imported *Trichogaster lalia* (Synonym for dwarf gourami) from Singapore^[20] and in *Anabas testudineus* (climbing perch), *Channa striata* (striped snakehead) and snakehead murrel in Vietnam^[21]. *Heteronchocleidus stunkardi* and *H. asymmetricus* have been identified in 85% and 4% of *Colisa lalius* in India, respectively^[21,22].

It has been suggested that monogeneans have significantly high host specificity^[23] which is expressed as the presence of at least one species on every species of certain host groups. Perhaps the qualitative and quantitative distinction in the patterns of host specificity of monogeneans contributes to the disparity between the sizes of the monogenean super-genera^[1]. From the *Dactylogyrus* checklist, it has been calculated that each species parasitizes a mean number of about 1.45 host genera; 78% of the species are reported from a single genus^[24].

Parasites imported with dwarf gourami fish may present risks, both to native fish populations and to the aquaculture industry and consequently can cause serious outbreaks of disease. In addition to the lesions in gills and skin, rapid and easy transmission can happen that indicates the necessity of quarantine, prevention, and treatment. Therefore, fish density level during export is so important to prevent such parasite infections via fish to fish transmission. It is recommended that the fishes should be examined and treated before domestic distribution and quarantine practices should be implemented. Further studies are required to determine the prevalence of parasitic pathogens in imported fishes into Iran.

ACKNOWLEDGMENTS

The author would like to thank Dr. Mehrdad Ameri and Dr. Shahram Rabbani (Tehran Heart Center; Tehran University of Medical Sciences) for help in preparation and review of this manuscript.

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