Helminthes parasite Isolated from a cyprinid fish, (*Capoeta barroisi* (Lortet, 1894)) in Dalaki River, Boushehr province, Iran

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Abstract

This study was conducted to identify intestinal helminthes. The helminthes were collected from body cavity and intestines of 100 specimens of *Capoeta barroisi* which were obtained from Dalaki River and investigated between July of 2012 and April of 2013. Most of the 3 species of helminthes were found in the intestine and body cavity of the examined fishes. The helminthes found composed of 2 Nematodes which have been reported previously. A total of 50 parasites were found in the 26 infected fish. The observed nematode and acanthocephalan parasites were identified to genus and species level respectively *Rhabdochona* sp. *Contracaecum* sp. *Neoechinorhynchus zabensis*. Most parasite species found in this study have been reported for the first time in this fish species from Iran. These inland water parasites can infect other freshwater aquatic animals and even human, so identification of them is important for health centers and fisheries research. In this study prevalence of parasites were identified respectively 4,5and 23 and Mean intensity were 1, 1.5 and 1.7 was reported

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Introduction

The most important and richest family of fish is Cyprinidae, the genus *Capoeta* of Cyprinid fishes inhabit mainly fast flowing streams and rivers of the Levant, Middle East, Caucasus and Southwestern Asia, but some species may also be found in lakes and springs (Coad, 1995). *Capoeta barroisi* (Lortet, 1894), one of the species of the genus *Capoeta* inhabits South of Iran. Ecologically, this species can be found on the sandy, pebble and Gravel-bed Rivers and streams. This fish feeds of bottom-living organisms, insect larvae, aquatic plants, benthos, algae clinging to the river bed (on the rocks and stones) and some aquatic insects such as Chironomida (Abdoli, 1999). The distribution areas of this fish is Eastern Mediterranean watersheds (Friche,

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Bilecenoglu & Sari 2007), Tigris- Euphrates basin, middle and lower Helleh, lower Mond, and lower Dasht-e Palang rivers in the Gulf basin (Coad, 2010).

The Helleh basin comprises rivers that begin in Zagros Mountains and flows southwest to the north of the Persian Gulf (Soltani, Kakoolaki & Kisami 2000). The Helleh watershed comprises the Dalaki, Shahpur and Helleh rivers and covers about 20,300 km2 and includes Lake Famur. (Coad, 2010). Dalaki River is a permanent freshwater river, close to the Dashtestan City (latitude: 29°28' and longitude: 51°17'), with 115 kilometers length in the Bushehr province and average depth of 70 cm (Bibak et al., 2012) in south Iran which is situated in Mesopotamian sub region, Gulf basin.

There are 140 species belonging to 24 families that make up the inland water fish of Iran (Abdoli, 1999). In spite of this biological richness, however, these populations are facing problems and even extinction due to suddenly ecosystems changes. Fish diseases are one of the harmful factors that cause losses and mortality (Aydoġdu, Emre, Y, Emre, N, & Altunel 2011). Helminth parasites cause severe mechanical damage due to high mobility, some of them are zooneoses and vector or reservoir of microorganisms, so study of these fishs can be important and useful considerably parasite life cycle, transportation to another population and ecologically helminth parasites of endemic fish species.

About 64 years ago, the first parasitological article was published by Bychowsky (1949), who reported four parasites on the gills of Iranian freshwater fishes in Karkheh River. The first record of genus *Rhabdochona (Nematoda)* was in intestine of *Capoeta capoeta gracilis* from Sefid Rood River in Gilan Province reported by Mokhayer (1980). *Rhabdochona fortunatowi* in intestine of *capoeta capoeta gracilis* from the Shiroud River in the Caspian drainage basin was reported (Golestaninasab, Malek, Jalali & Mobedi 2012; Pazooki, Nazari Chamak, &Masoumian 2012) identified *Rhabdochona denudata* and *Rhabdochona macrostoma* from *Capoeta damascina*, other researchers that reported *Rhabdochona* from genus Capoeta in Iran include (Pazooki and Masoomian 2001; Fadaei, Mokhayer, & Ghorbani, 2001;

Peyghan, Nabavi, & Hoseini 2004 ; Aydoĝdu et al 2011) isolated *Rhabdochona denudata* from *C. antalyensis* in Turkey, Bilal and Abdulla (2009) reported *Rhabdochona gnedini* and *Rhabdochona tgraei* from *Capoeta damascinus* in Iraq. The first report of *Contraceacum* sp. (Nematoda) in gastrointestinal tract from *Capoeta capoeta gracilis* of Aras River in West Azerbaijan province, Iran was done (Pazooki and Sayar 2000). Another report of *Contraceacum* sp was presented by Jalali jafari and Miar (2011) and Johargholizadeh (2006). Bagherpour, Afsharnasab, Mobedi, Jalali, & Mesbah 2011) reported some helminthes infected in Black sole fish, from Persian Gulf.

In term of Acanthocephal infections reported in Iranian inland waters, the first

information on *Neoechinorhynchus sp.* (Acanthocephala) from the genus *Capoeta* was given by Mokhayer (1980), later infected cases have been reported from genus *Capoeta* by Peyghan et al. (2004 ; Pazooki et al. 2005 and Johar-gholizadeh 2006). Oğuz, Amin, Heckmann, Tepe, Johargholizadeh, Aslan, & Malek, (2012) discovered new morphological features of *Neoechinorhynchus zabensis* from *Capoeta damascina* and *Capoeta trutta* in Turkey, Bilal and Abdulla (2009) also found this species in the intestine of *Capoeta trutta* and *Varicorhinus umbla*. Firstly genus *Acanthocephalorhynchoides* (Acanthocephala) studied by Williams, Gibson & Sadeghian (1980) from genus *Capoeta* in Iran, after that Fadaei et al. (2001) and Bozorgnia, Youssefi, Barzegar, Hosseinifard, & Ebrahimpour, (2012) isolated this genera from genus Capoeta in Iran inland waters. Due to the restricted distribution of the genus Capoeta in the Asian region, such as Iran, Iraq and Turkey, parasitologically insufficient investigation has been carried out on these fish genera. To the authors' knowledge, there is no record of Ichthyoparasitological data for *Capoeta barroisi.*

The aim of the present paper was to provide information on the parasito coensis, to discuss the parasitofauna of endoparasites, biodiversity of parasites and to introduce the fish metazoan parasites composition in Dalaki River. Little attention was paid to parasites of *Capoeta barroisi* of Dalaki River ecosystem.

Materials and Methods

This study was conducted between July of 2012 and April of 2013 in tributaries of the Persian Gulf basin. After determining the study station in Dalaki River, sampling was carried out by using a cast net. (Fig. 1)

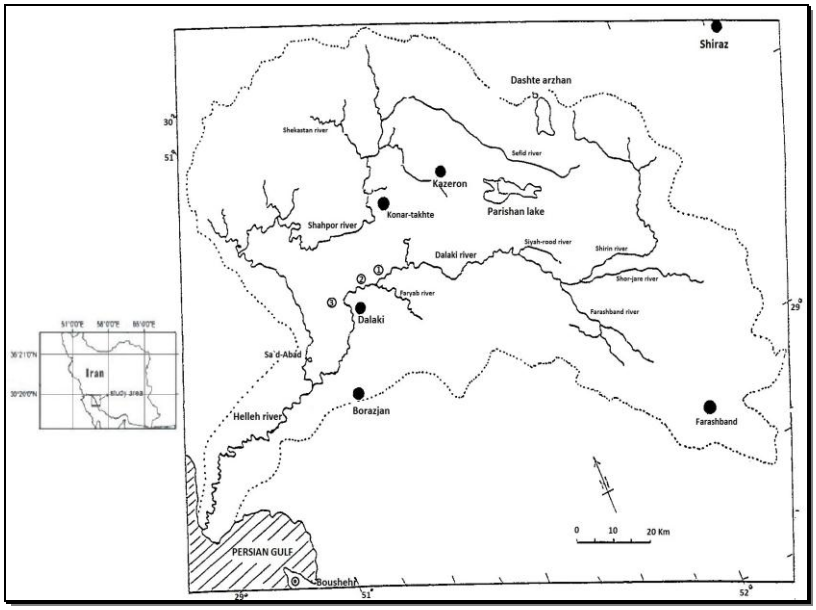

Figure 1 Map of the capture region

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A total of 100 *Capoeta barroisi* were collected from this river (Fig. 2). These caught fish were kept on ice and transported to the laboratory of Aquatic Department, Boushehr branch of Islamic Azad University. Host fish were identified based on Abdoli (1999) and Coad (1992 & 1995) Iranian ichthyologist identification key. The standard length and weight of each fish was measured to the nearest millimeter and milligram, the sex was determined internally (Fig. 2).

Figure 2 *Capoeta barroisi*

Fig. 3 Dissection of examined fish, *Capoeta barroisi*

The methods and techniques used for collection, relaxation, fixation, staining and mounting of helminthes are basically those described by Hanek and Fernando (1972) and Roberts (2001).

Fish were examined for the presence of ectoparasites then dissected and internal studied for cysts and endoparasites in the muscles and visceral organs. Abdominal cavity of each fish was washed with a 0.6% saline solution for abdominal parasites. Then the gastrointestinal tract was dissected for

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examination. Intestine cut open longitudinally, the contents were washed was removed, placed in a petri dish, and examined for helminthes using an Olympus SZ Series 51 stereomicroscope.

Nematodes were washed in saline (0.6%-0.8%) and fixed in hot 76% ethyl alcohol and cleared in Glycerin or in hot lacto phenol. For permanent preservation mounted on azocarmin stains. All Nematodes were identified. Using descriptions identification keys Yamaguti (1961), Gussev (1985) and Moravec (1994). Acanthocephalans were isolated from dissected Fish hosts then fixed in 70% ethanol then stained in Mayer's carmine or azocarmin. After staining, dehydrated in alcohol series, for clearing were placed in xylol and finally mounted in Canada balsam. Then the parasite was identified, according to morphological characteristics and followed the key to the families and subfamilies of Acanthocephala (Amin, 1987). All isolated nematodes and acanthocephalans specimens in each individual fish were identified and counted. A camera Lucida was used to draw parasites and photographs were taken by a Canon digital camera (A1000).

Results

During the current survey, from a total of 100 examined fish, 26 specimens of *C. barroisi* collected over a period of 12 months, were infected with various parasites. A total of 50 parasites were found in the 26 infected fish. (Tables 1 & 2)The observed nematode and acanthocephalan parasites were identified to genus and species level respectively.

Table 1 Prevalence of internal parasites in examined *Capoeta barroisi* fish in Dalaki River

	Fish infected	Fish uninfected	Prevalence (%)
Rhabdochona sp.	4	96	4
Contracaecum sp.	5	95	5
Neoechinorhynchus zabensis	23	77	23

According to the Duncan test (sig < 0.05); there were significant differences between spring and summer season and autumn and winter seasons (Table 2).

Table 2 Seasonal prevalence of different helminthes in *Capoeta barroisi* fish in Dalaki River

	Spring (%)	Summer (%)	Autumn (%)	Winter (%)
Rhabdochona sp.	12	4	0	0
Contracaecum sp.	12	8	0	0

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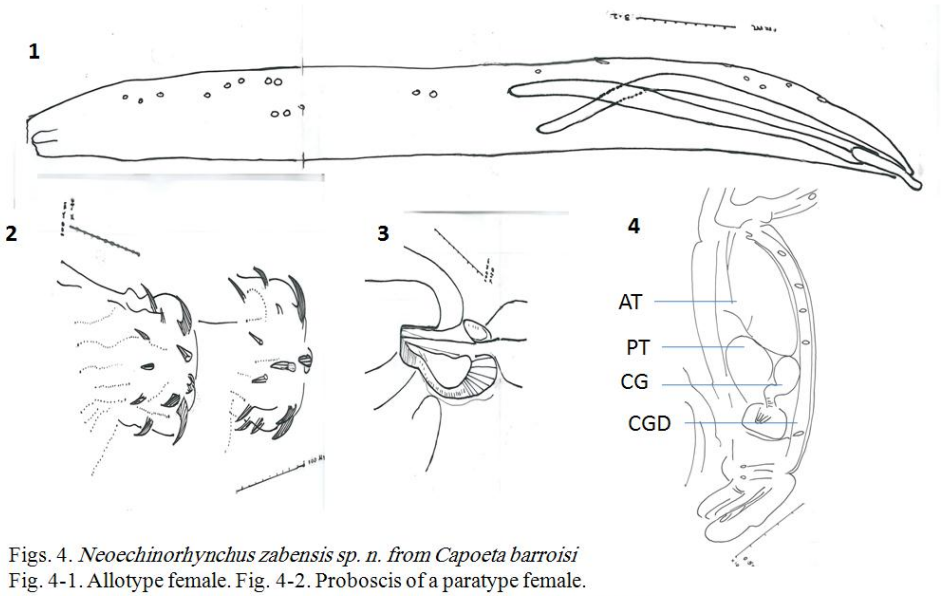
Neoechinorhynchus zabensis	44	36	0	12

Table 3 Intensity of parasites in *Capoeta barroisi* fish in Dalaki River

	The lowest No. of parasites in infected fish	The highest No. of parasites in infected fish	Mean intensity
Rhabdochona sp.	1	2	1
Contracaecum sp.	-	1	1.5
Neoechinorhynchus zabensis	1	3	1.7

Neoechinorhynchidae, Neoechinorhynchinae with characters of the genus and subgenus *Neoechinorhynchus*. Trunk medium, cylindrical with sexual dimorphism in size of all common structures. Trunk with thick walls not dorso-ventrally distinguished.



Figs. 4. *Neoechinorhynchus zabensis sp. n. from Capoeta barroisi*
Fig. 4-1. Allotype female. Fig. 4-2. Proboscis of a paratype female.
Fig. 4-3. Reproductive system of a paratype male.Fig.4-4. AT –anterior testis; CG – cement gland; CGD – cement gland duct; PT – posterior testis

Neoechinorhynchus have ten dorsal large nuclei and three ventral hypodermal mega nuclei (Fig 4-1). Proboscis as long as apical organ. Anterior hooks largest than other worm, with simple roots. Hooks in second circles slightly shorter than anterior hooks. Most of hooks in second circle longer than hooks in third circle. Neck unremarkable. Proboscis receptacle about 5- 6 times as long as proboscis, with single muscular wall in anterior half. Cerebral ganglion large, between circle and oval and as long as proboscis, at base of receptacle (Fig 4-2 to 4-4). Morphometric characteristics of *N. zabensis* are shown in Table 4. According to Table 1

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overall prevalence was 23% and average intensity was 1.7 specimens per fish (Table 3). *Rhabdochona sp.* had relatively short body with a smooth cuticle; tail of both sexes had sharp cuticular spike ends. Spicules were different (Table 4). This nematode was found in fishes' intestines. Prevalence was 4% (Table 1) and according to Table 3 the mean intensity was one parasite in fish.

Table 4 Measurements of *Rhabdochona* sp.

Rhabdochona sp.	Measurement mean (mm)
Male Body length	7.65
Female Body length	8.22
Number of Caudal pappilles	10
Large Spicule	0.432
Small Spicule	0.178
Muscular esophagus	0.371
Glandular esophagus	3.095

Length of *Contracaecum sp.* was 8.1 mm, and average width was 0.23 mm. Esophagus length of this genus was 0.4 mm. In the present study the general prevalence was 5% (Table 1) and according to table 3 the mean intensity was 1.5 nematodes per fish.

Discussion

Neoechinorhynchus has a wide spread among fishes in Iraq as 15 species of fresh water fishes were records as definitive hosts for this genus in Iraq (Mhaisen 2002). Between parasites that cause infection in fish of freshwater, brackish-water and marine environments throughout the world, nematodes are of particular importance because they cause mechanical and nutritional deficiencies in the host. Present knowledge of these parasites still remains incomplete, especially those pertaining to biology and ecology, but also taxonomy, phylogeny and zoogeography (Moravec 2007).

Neoechinorhynchus zabensis is distinguished from all other species of the genus by having a paired muscular para-vaginal appendage. We found no records of nuclear fragments in the lemnisci or the structure present in the proboscis receptacle wall in the description of any of the other 88 species of the genus (Amin, Abdullah, & Mhaisen 2003). Oğuz et al. (2012) observed *N.zabensis* features in *Capoeta barroisi* included: size of anterior hooks at the first and second levels had been different length, middle and posterior hooks at close position had been similar length.

Whereas *N. zabensis* shows higher prevalence in males than females and juveniles, there was no record of any

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acanthocephalan infection **(**Koyun, 2012). Aydoğdu et al. (2011) reported *Rhabdochona denudata* and *Contracaecum* sp. larvae from *C. antalyensis* and *P. battalgil*, respectively. This study has revealed that freshwater *Capoeta barroisi* from Boushehr province in Iran were infected with the following two nematodes and one acanthocephalan species: *Rhabdochona sp.*, *Contraceacum sp.* and *Neoechinorhynchus zabensis.*

Rhabdochona sp was originally described by Moravec (1994) from the intestine of *Varocorhinus capoeta* in freshwater in Azerbaijan. So this is the first record of an observation in Iran.

In conclusion, the results show that varieties of nematodes and one acanthocephalan were distributed in different ecosystems and climates, as well as in zoogeographic features of native fishes. It would be interesting to consider parasites may survive in different climates.

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جداسازی انگلهای کرمی از کپور ماهی شکل(*Capoeta barroisi* (Lortet, 1894 در رودخانه دالکی استان بوشهر، ایران

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چکیده

هدف از این پژوهش بررسی انگلهای کرمی داخلی ماهی آب شیرین *Capoeta barroisi* در استان بوشهر بود. بر این اساس تعداد ۱۰۰ نمونه از این ماهی از رودخانه دلکی واقع در استان بوشهر در تابستان سال ۱۳۹۱ تا بهار سال ۱۳۹۲ به تدریج جمع آوری گردید و پس از بررسی های ظاهری، انگلهای محوطه شکمی و روده نمونه ها شستشو و مورد مطالعه جهت شناسایی قرار گرفت. در این مطالعه سه جنس و گونه از کرمهای انگلی داخلی مورد شناسایی قرار گرفت که عمدتا در محوطه شکمی و روده این ماهی حضور داشتند، دو مورد از کرمها نماتد بود که قبلا نیزگزارش شده بودند و یک مورد از کرمهای آکانتوسفال گزارش گردید که بترتیب کرمهای رابدوکانا ، کنتراسکوم و نئواکینورینکوس زابینسیس گزارش شد این انگلها برای اولین بار از این گونه ماهی در ایران گزارش گردیده است. در این مطالعه میزان شیوع این انگلها به ترتیب ۴، ۵ و ۲۳ و میانگین شدت آلودگی ماهی به انگلها به ترتیب ۱/۵،۱و ۱/۷ گزارش می شود.

کلمات کلیدی: ماهیان آب شیرین، انگلهای کرمی، انگل آکانتوسفال و ایران

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