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FIRST REPORT OF *Neoechinorhynchus rutili* IN *Cobitis faridpaki* (Cobitidae) FROM THE SOUTHERN CASPIAN SEA BASIN

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ABSTRACT

Members of the Cobitidae family are not economically important, thus few studies have been made on their parasitology and there is not a single report on their parasites in Iran. In the present study, a total of 225 individuals of *Cobitis faridpaki* were collected from the Babolrud River around the southeast of the Caspian Sea basin (Mazandaran province, north of Iran) on 12 separate occasions between May 2009 and April 2010**.** After recording biometric characteristics, common necropsy and parasitology methods were used. In this study, a total of 193 individuals of an acanthocephalan parasite, *Neoechinorhynchus rutili* were found in *C. faridpaki.* The prevalence, mean intensity of infection ± standard deviation (SD), range and mean abundance ± SD of the parasite are as follows, respectively: 63.11, 1.36±0.61, 1-4 and 0.86±0.82. The prevalence, mean intensity of infection and abundance of *N. rutili* were significantly different in various age classes, but it was not the case for different seasons and the sexes. The occurrence of *N. rutili* is reported for the first time from *C. faridpaki* in Iran.

INTRODUCTION

Cobitidae, a family of loaches, sometimes called sting-loaches, is found in Eurasia and Morocco and has about 26 genera with about 177 species (Nelson, 2006). The genus *Cobitis* Linnaeus, 1758 is one of the most widely distributed Palearctic primary freshwater fish groups (Nalbant et al., 2001). The *Cobitis* genus fishes are represented in Iran by three valid species: *C. linea* Heckel, 1849, *C. faridpaki* Mousavi-Sabet, Vasil'eva, Vatandoust and Vasil'ev, 2011 and *C. keyvani* Mousavi-Sabet, Yerli, Vatandoust, Ozeren and Moradkhani, 2012. *C. linea* is found in the Kor River basin, central Iran and *C. faridpaki* and *C. keyvani* are found in the south of the Caspian Sea basin (Mousavi-Sabet et al., 2011b, 2012d). Despite their wide distribution in Iranian basins, these species have been poorly investigated (Mousavi-Sabet et al., 2011a, 2012b, 2012c).

Parasites of bonyfish species in the Caspian Sea and its basin have been reported by several authors (Eslami and Kohneshahri, 1978; Sattari, 1996, 1999; Pazooki and Aghlmandi, 1998; Sattari et al., 2002, 2005; Daghigh Roohi and Sattari, 2004; Khara et al., 2005, 2011), but as the members of the Cobitidae family are not economically important, except sometimes in aquarium trade (Mousavi-Sabet, 2012a), few studies have been made on their parasitology.

Neoechinorhynchus species are acanthocephalan with delicate slender body. Hypoderm contains small and generally fixed number of oval giant nuclei. Proboscis is small and spherical with few longish hooks in six spiraling rows, three hooks per row. One large rooted hook is located in each spiral row, two other hooks are considerably smaller and rootless. Lemnisci are of approximately equal length or slightly different. Ganglion of central nervous system is located in the lower part of proboscidal sheath. Eggs are oval, with thin oval shells (Bykhovskaya-Pavlovskaya et al., 1962).

Neoechinorhynchus species are found in intestine of various freshwater fishes (about 60 species), most often Cyprinids and Salmonids, occasionally Perches, Cod, Graylings, Pikes, Gobiid and Cottid Gobies and Sculpins, almost ubiquitous, including Far East. *Neoechinorhynchus* species can cause lesions of the intestinal mucosa in mass infestations. Cases have been described of trout yearling fatalities (Bykhovskaya-Pavlovskaya et al., 1962).

In the present study, however, attempts were made to study the parasite composition and communities of *C. faridpaki* in the Babolrud River around the southeast of the Caspian Sea (Mazandaran province, Iran), as well as epizootiological aspects through calculating prevalence, intensity and abundance.

MATERIALS AND METHODS

A total of 225 individuals of *C. faridpaki* were collected from the Babolrud River in the southeast of the Caspian Sea basin (Mazandaran province, Iran) on 12 separate occasions between May 2009 and April 2010**.** Fish were captured by electrofishing and transported to the laboratory of Fish Diseases in the Faculty of Natural Resources, University of Guilan, Iran. Water temperature was determined at the collection site. Upon arrival, fish were weighed and measured and then examined externally for gross signs of parasitism. *C. faridpaki* (225 in number) averaged 1.85 g (±1.05 g, range=0.25-6.18 g) in weight and averaged 56.42 mm (±12.88 mm, range=27.00-92.50 mm) in total length. A gill biopsy was collected from the second left arch of the specimens. A fin biopsy was collected from the caudal fin. Wet mounts of all biopsied tissues were prepared for further analysis.

After recording biometric characteristics, common necropsy and parasitology methods (Stoskopf, 1993) were used. All organs of the fish were examined except for blood. Live acanthocephalans were relaxed in distilled water at 4°C for 1 h and fixed in 10% hot buffered formalin. All specimens were stained with aqueous acetocarmine, dehydrated and mounted in Permount. The worms were identified using parasite identification keys (Yamaguti, 1961; Bykhovskaya-Pavlovskaya et al., 1962; Moravec, 1994) and then deposited at the Laboratory of Fish Diseases, Faculty of Natural Resources, University of Guilan, Iran.

Statistical analysis. Classical epidemiological variables (prevalence, intensity and abundance) were calculated according to Bush et al. (1997). Mean intensity of infection was determined dividing the total number of recovered parasites by the number of infected fish samples, while calculating abundance was carried out dividing the total number of recovered parasites by the number of (infected and uninfected) fish samples. Prevalence was also calculated dividing the number of infected fish samples by the total number of examined ones and expressed as a percentage. Mean intensity of infection and abundances of parasite species (with prevalence >10%) among seasons, age classes and the sexes were tested by the Kruskal-Wallis test (KW, multiple comparisons) and Mann-Whitney U test (MW, pairwise comparisons). Results were considered significant at the 95% level ($P<0.05$). Computations were performed using the SPSS version 16 software package and Microsoft Office Excel 2010.

RESULTS

In the present study, a total of 193 *Neoechinorhynchus rutili* (Figures 1 & 2) were found in *C. faridpaki.* The occurrence of *N. rutili* is reported for the first time from *C. faridpaki* in Iran. The prevalence (P), mean intensity of infection (MI), range and mean abundance (MA) of the parasite are as follows: P= 63.11, MI= 1.36±0.61, 1-4 and MA= 0.86±0.82.

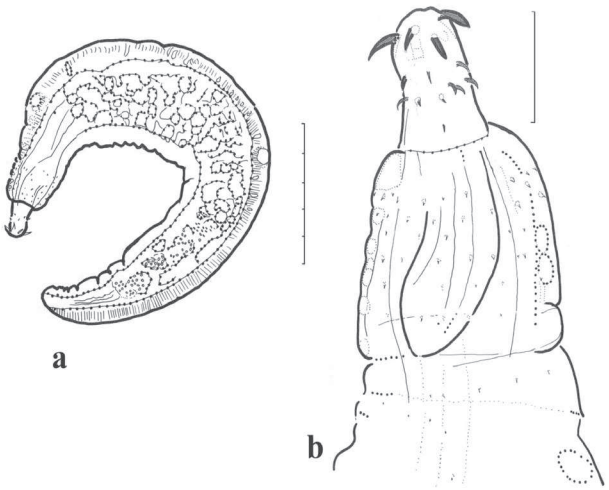

Fig 1. *Neoechinorhynchus rutili* infecting *Cobitis faridpaki.* (a) lateral view of a male, scale-bar: 500 µm; (b) proboscis of mature male, scale-bar: 100 µm

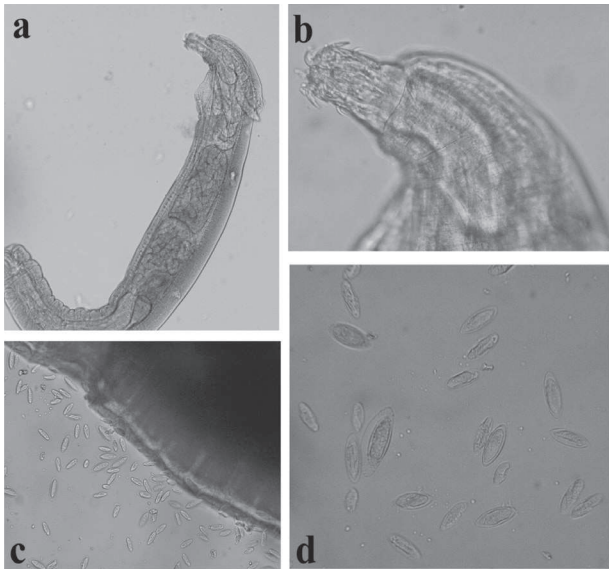

Fig 2. (a) *Neoechinorhynchus rutili* from *Cobitis faridpaki* intestine, mag. X100; (b) Spicule of *N. rutili* infecting *C. faridpaki*, mag. x400; (c and d) The eggs of *N. rutili*, mag. X200 and X400

The prevalence (P), mean intensity of infection (MI), range and mean abundance (MA) of the parasites in different seasons, sexes and age classes are presented in Tables 1-3.

As shown in Table 1, the prevalence of *N. rutili* was different in various seasons, but the differences were not significant (Z test, p>0.05). It was also true for mean intensity and abundance of this parasite in different seasons (KW test, $X^2 = 1.839$, df=3, p>0.05 for mean intensity and $X^2 = 1.823$, df=3, p>0.05 for abundance).

As shown in Table 2, the prevalence of *N. rutili* was different in various sexes, but the differences were not significant (Z test, P>0.05). It was also true for mean intensity and abundance of this parasite in different sex classes (MW test, $X^2 = 0.058$, df=1, P>0.05 for mean intensity and $X^2 = 0.819$, df=1, P>0.05 for abundance).

As shown in Table 3, the prevalence of *N. rutili* was different in various age classes and the differences were significant (Z test, P<0.05). It was also true for mean intensity and abundance of this parasite in different age classes (KW test, $X^2 = 26.239$, df=4, P<0.05 for mean intensity and $X^2 = 50.102$, df=4, P<0.05 for abundance).

In the present study, it was found that the mean intensity and abundance of *N. rutili* in various weight classes had different values, and the differences were significant (KW test, $X^2 = 20.117$, df=5, P<0.05 for mean intensity and $X^2 = 36.838$, df=5, P<0.05 for abundance).

It was also found that the abundance of *N. rutili* in various length classes had different values, and the differences were significant (KW test, $X^2 = 81.628$, df=55, P<0.05), but it was not true for the mean intensity of *N. rutili* in different length classes (KW test, $X^2 = 58.3$, df=47, P>0.05).

Table 1. The prevalence, mean intensity of infection, range and abundance of *Neoechinorhynchus rutili* in *Cobitis faridpaki* (N = 225) in different seasons

Season	No. of fish	Prevalence (%)	Mean ±SD	Range	Abundance ±SD	No. of parasites
Spring	51	68.33	1.37±0.73	1-4	0.94±0.88	48
Summer	51	60.78	1.32±0.54	1-3	0.80±0.78	41
Autumn	61	62.30	1.42±0.55	1-3	0.89±0.82	54
Winter	62	61.29	1.32±0.62	1-3	0.81±0.81	50

Table 2. The prevalence, mean intensity of infection, range and abundance of *Neoechinorhynchus rutili* in *Cobitis faridpaki* (N = 218) in different sex groups

Sex	No. of fish	Prevalence (%)	Mean ±SD	Range	Abundance ±SD	No. of parasites
Male	88	60.23	1.32±0.51	1-3	0.80±0.76	70
Female	130	66.15	1.40±0.67	1-4	0.92±0.86	120

Table 3. The prevalence, mean intensity of infection, range and abundance of *Neoechinorhynchus rutili* in *Cobitis faridpaki* (N = 225) in different age groups

Age	No. of fish	Prevalence (%)	Mean ±SD	Range	Abundance ±SD	No. of parasites
1+	34	38.24	1.0±0.0	1	0.38±0.49	13
2+	89	53.93	1.17±0.38	1-2	0.63±0.65	56
3+	82	74.39	1.39±0.59	1-3	1.04±0.79	85
4+	20	100	1.95±0.89	1-4	1.95±0.89	39

DISCUSSION

Members of the Cobitidae family are not economically important, thus few studies have been made on their parasitology. Data on freshwater fish diseases are limited mostly to commercially important species. Loaches are hosts to numerous parasites and are affected by various diseases, as well as important secondary hosts for certain parasites (Koster et al., 2002). The presence of disease agents may influence the structure and size, feeding habits, growth rates and reproduction of loach populations.

To date, there are no records of parasites of the *Cobitis* species in Iran. However, future research should comprise more regular sampling, with higher numbers of particular species.

Parasites affect almost every conceivable level of biological organisms. Generally, the importance of these abundant, species-rich and wide-spread life forms is not well known (Lindstrom et al., 1994). Recent research has also revealed that the species richness of *Cobitis* characterized by geographic diversity has established scientific interest in all aspects of their biology. Some of the species in this genus are considered as vulnerable to endangered, according to IUCN (1990) methodology. The question then arises as to what extent the presence of parasites influences the vulnerability or abundance of a particular species (Zrncic et al., 2009).

The ectoparasites were not found in the present study. Also, few studies have been published on the parasite fauna of the representatives of the genus *Cobitis*. As to literature data, most scientific papers deal with the description of endoparasitic helminths and the monogenean *Gyrodactylus cobitis* of different *Cobitis* species (Bychovskij, 1962; Bauer, 1984; Moravec, 2001; Moravec, 2001; Popiolek and Kotusz, 2004). Robotham and Thomas (1982) reported the digenean parasite *Allocreadium transversale* in the intestines of *C. taenia*. Bychovskij (1962) and Bauer (1984) listed parasites observed in the representatives of the genus *Cobitis* comprising parasitic protozoa and metazoa. The high intensity of infection in *C. elongatoides* by Plerocercoids of the tapeworm *Ligula colymbi* as well as metacercariae of the trematode *Posthodiplostomum cuticula* in hybrids of *C. elongatoides* x *Cobitis* sp. in the Czech Republic was observed by Halacka et al. (2000). Parasitological examination of *C. elongatoides* and hybrids of *C. elongatoides* and *C. taenia* in Poland revealed infections by six trematode species (*Diplostomum* sp., *Echonostomatidae* gen. sp., *Tylodelphys clavata*, *Posthodiplostomum cuticula*, *Metorchis xanthosomus* and *Allocreadium transversale*) and the nematode *Rhabdochona ergensi* (Popiolek and Kotusz, 2003, 2004; Popiolek et al., 2003). Scholz et al. (2004) reported the presence of the cestode *Neogryporhynchus cheilancristrosus* in *C. taenia* and *Misgurnus fossilis*. A survey of parasitic fauna of some representatives of the loaches, such as *C. elongata*, *C. elongatioides* and *C. bilineata*, was carried out at four Croatian rivers by Zrncic et al. (2009) and six parasite species were found including the ciliate parasites *Trichodina nigra* (61.29%), *Epistylis* sp. (12.90%), *Chilodonella cyprini* (22.58%), *Ichthyophthyrius multifiliis* (81.81%), dinoflagellate *Piscioodinium pillulare* (6.45%) and monogenean *Gyrodactylus cobitis* (22.22%). In the present study, the mean intensity of infection was low, so no obvious sign of lesions in intestinal mucosa was observed.

Sažetak

PRVI NALAZ O POJAVI PARAZITA *Neoechinorhynchus rutili* KOD PORODICE *Cobitis faridpaki* (Cobitidae) U JUŽNOM DIJELU KASPIJSKOG JEZERA

Vrste iz porodice Cobitidae nisu gospodarski važne, o čemu svjedoči tek nekolicina studija o njihovoj parazitologiji i nedostatak bilo kakvog izvješća o njihovim parazitima u Iranu. U ovom je istraživanju prikupljeno ukupno 225 jedinki *Cobitis faridpaki* iz rijeke Babolrud u jugoistočnom dijelu Kaspijskog jezera (pokrajina Mazandaran u sjevernom Iranu) tijekom 12 navrata od svibnja 2009. do travnja 2010. godine. Nakon bilježenja biometrijskih karakteristika, primijenjene su uobičajene metode autopsije i parazitologije. U ovom je istraživanju pronađeno ukupno 193 primjeraka parazita akantocefala (*Neoechinorhynchus rutili*) kod vrste *C. faridpaki*. Učestalost, srednji intenzitet zaraze ± standardno odstupanje (SD), raspon i srednja brojnost ± SD su iznosili 63,11, 1,36±0,61, 1-4, odnosno 0,86±0,82. Učestalost, srednji intenzitet zaraze i brojnost *N. rutili* bili su znatno drugačiji u različitim dobnim skupinama, ali ne i za različita doba godine i spolove. Pojava *N. rutili* prvi put je zabilježena kod vrste *C. faridpaki* u Iranu.

Ključne riječi: Cobitidae, *Cobitis faridpaki*, parazit, Kaspijsko jezero, rijeka Babolrud

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