Occurrence and intensity of parasites in Prussian carp, *Carassius gibelio* from Anzali wetland, Southwest Caspian Sea

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Abstract

The aim of this study was to detect the occurrence of parasites in Prussian carp, *Carassius gibelio* as the most important alien fish in Anzali international wetland. This undesirable fish was introduced accidentally to Iran with Chinese carp fries which imported to the country during the last decades and then acceded to Anzali wetland. Today this fish has significant stocks in Anzali wetland; but there have been limited studies about the parasites of this fish in Anzali wetland. During this study a total of 90 Prussian carp were collected by electrofishing and gillnets from April through July 2012. After recording biometric characteristics, common necropsy and parasitology methods were used. A total of 2715 individuals out of 11 parasite species were recovered. Parasitofauna consisted of: two protozoans, *Ichthyophthirius multifiliis* and *Trichodina* sp.; one nematode, *Raphidascaris acus*; one trematode, *Diplostomum spathaceum*; six monogeneans, *Dactylogyrus formosus, Dactylogyrus dulkeiti, Dactylogyrus baueri, Dactylogyrus arquatus, Dactylogyrus inexpectatus and Gyrodactylus kobayashii*; and one crustacean, copepodid stage of *Lernaea cyprinacea*. The monogeneans had the highest prevalence values (88.89%). The occurrence of *D. inexpectatus* in *C. gibelio* is reported for the first time in Iran.

Keywords: Prusian carp, *Carassius gibelio*, parasites, Anzali wetland, Caspian Sea

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Introduction

Anzali international wetland, with a surface area of about 37,065 acres and 49 fish species is located in the Southwest of Caspian Sea. One of its alien habitant is *Carassius gibelio*. The fish inhabits a wide variety of still water bodies and lowland rivers, usually associated with submerged vegetation or regular flooding. It can strongly tolerate low oxygen concentrations and pollution. This fish imported to Iran along with other species of fish by accident during the few last decades.

Parasites of bony fish species in the Caspian Sea and its basin have been reported by several authors (Eslami and Kohneshahri, 1978; Sattari, 1996; Daghigh Roohi, 1997; Sattari, 1999; Sattari et al., 1999; Pazooki and Aghlmandi, 1998; Daghigh Roohi and Sattari, 2004; Sattari et al., 2005; Khara et al., 2005; Khara et al., 2011), but there have been limited studies on Prussian carp parasites in the study area. Sattari (1996) reported 4 parasite species from Prussian carp consisting of *Raphidascaris acus, Diplostomum spathaceum,* larvae, *Dactylogyrus extensus* and *Gyrodactylus* sp. in Anzali weland. In the other studies, six monogenean species including *Dactylogyrus formosus, Dactylogyrus baueri, Dactylogyrus extensus, Dactylogyrus vastator, Dactylogyrus wegeneri* and *Gyrodactylus prostae* reported from Prussian carp in Iran (Jalali and Molnar, 1990; Jalali, 1995; Shamsi and Jalali, 1997). In addition, Khara et al. (2005) recovered two parasite species from Prussian carp consisting of larvae of *Diplostomum spathaceum* and a *Dactylogyrus* sp. in Amirkelayeh wetland (37º 17´ N, 50º 12´ E). Khara et al. (2011) also found these two parasite species from Prussian carp in Boojagh wetland (37º 27´ N, 49º 55´ E), but there is no recently published report about the parasite communities of Prussian carp and epizootiological aspects of these parasites in Anzali wetland.

In the present research, attempts were made to study the parasite composition and communities of *C. gibelio* in Anzali wetland as well as their epizootiological aspects through calculating their prevalence, intensity, abundance and dominance.

Materials and methods

A total of 90 Prussian carp, *C. gibelio* were collected from Anzali international wetland (37º 25´ N; 49º 28´ E) in the southwest of the Caspian Sea (Guilan province, Iran) on 6 separate occasions from April through July 2012. Fish were captured with gillnets and transported to the fish disease laboratory of National Inland water Aquaculture Research Institute in Anzali city, alive in water obtained from the collection site. A dissolved oxygen saturation of approximately 85 - 90% was maintained during transport. Water temperature was determined at collection site. Upon arrival, fish were weighed, measured and then a few scales picked up for age determination. Fish was examined externally for gross signs of parasitism. If no gross signs were observed, skin biopsies were prepared from the entire length of the lateral body wall. A gill biopsy was collected from the specimen's second arch. A fin biopsy was collected from the specimen's caudal fin. Wet mounts of all biopsied tissues were prepared for further analysis.

After recording biometric characteristics, common necropsy and parasitological methods according Stoskopf (1993) were used. All organs of the fish were examined except blood. Live trematodes were relaxed in distilled water at 4 ºC for 1 h and fixed in hot 10% formalin. Live nematodes were fixed in hot 70% ethanol and cleared in hot lactophenol. All specimens fixed in 10% formalin 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 they were deposited at the Laboratory of Fish Diseases, Faculty of Natural Resources, University of Guilan, Iran.

Classical epidemiological variables (prevalence, intensity and abundance) were calculated according to Bush et al. (1997). The dominance of a parasite species was calculated as N/N sum (where N = abundance of a parasite species and N sum = sum of the abundance of all parasite species found) and expressed as a percentage based on Leong and Holmes, (1981). The dominance values were used for classification of parasites as eudominant (>10%), dominant (5.1% - 10%), subdominant (2.1% - 5%), recedent (1.1% - 2%) and subrecedent (<1.0%) of given species (Niedbala and Kasparzak, 1993). Mean intensity of infection and abundances of parasite species (with prevalence >10%) among seasons, age classes and sexes were tested by the Kruskal-Wallis test (KW, multiple comparisons) and Mann-Whitney U test (MW, pair wise comparisons). Results were considered significant at the 95% level ($p<.05$). Computations were performed using the SPSS programme.

Results

In the present study, 90 specimens of Prussian carps were investigated by 112.4 ± 119.14g (range = 5 – 713 g) and 17.73± 6.5 cm (range = 7.3 - 35.5 cm) average weight and fork length, respectively.

A total of 2715 individuals of 11 parasite species consisting of two protozoans: *Ichthyophthirius multifiliis* Fouquet, 1876 (Fig. 1) and *Trichodina* sp. (Fig. 2); one nematodes larvae of *Raphidascaris acus* (Bloch, 1779) (Fig. 3); one digenean: metacercaria of *Diplostomum spathaceum* (Rud, 1819) (Fig. 4); six monogeneans: *Dactylogyrus dulkeiti* Bykhovsky, 1936, *Dactylogyrus arquatus* Yamaguti, 1942, *Dactylogyrus inexpectatus* Izumova, 1955, *Dactylogyrus formosus* Kulviec, 1927, *Dactylogyrus baueri* Gussev, 1955, *and Gyrodactylus kobayashii* Hukuda, 1940 (Figs. 5 to 14) ; and one crustacean: copepodid stage of *Lernaea cyprinacea* (Fig. 15) were recovered from the Prussian carp. The occurrence of *D. inexpectatus* in *C. gibelio* is reported for the first time in Iran.

Two fish (2.22%) proved to be free of parasite; 23 fish (25.56%) were infected with one parasite species; 25 fish (27.78%) with two species; 30 fish (33.33%) with three species; nine fish (10%) with four species; one fish (1.11%) with five species. Fish harbouring fewer than 10 parasites made up 38 specimens

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in number (42.22%); 12 fish (25.58%) had 10-20 parasites; 22 fish (24.44%) harboured 21-50 parasites; 16 fish (17.78%) had more than 50 parasites.

The eudominant parasites of the Prussian carp (Table 1) were monogeneans and a *Trichodina* sp. (Dominance = D = 73.04% and 12.67% respectively). The dominant parasites were *D. spathaceum* (D=6.52) and *I. multifiliis* (D= 5.49%). The subdominant parasite was *R. acus* (D = 2.2%) and subrecedent parasite was copepodid stage of *L. cyprinacea.*

The prevalence (P), mean intensity of infection (MI), range and mean abundance (MA) of the parasites are presented in Table 1. As shown in Table 1, monogeneans (including *D. formosus, D,dulkeiti, D. baueri, D. arquatus, D. inexpectatus and Gyrodactylus kobayashi*) was indicated the highest prevalence values (88.89%) in Prussian carp. The mean intensity of infection and abundance of these parasites (24.79 and 22.03, respectively) were also higher than the other ones. Prevalence, mean intensity of infection and abundance of *D. spathaceum* (58.89%, 3.34 and 1.97, respectively) and *I. multifiliis* (31.11%, 5.32, 1.66, respectively) were also high. Copepodid stages of *L. cyprinacea* had lower values of prevalence, mean intensity and abundance than the other parasites (2.22%, 1.5 and 0.33 respectively).

Table 1: The prevalence, mean intensity, range, abundance and dominance of parasites in *C. gibelio*

Parasite	Prevalence(%)	Mean ± SD	Range	Abundance±SD	Dominance(%)
*Diplostomum** N=177	58.89	3.34±4.17	1-27	1.97 ± 3.59	6.52
Trichodina N=344	15.56	24.75±26.53	2-65	3.82±13.53	12.67
Ichthyophthirius N=149	31.11	5.32±6.48	1-24	1.66±4.34	5.49
Monogeneans** N=1983	88.89	24.79±32.66	1-150	22.03±31.75	73.04
*Lernaea**** N=3	2.22	1.50±0.71	1-2	0.03±0.23	0.11
*Raphidascaris** N=59	27.78	2.36±2.53	1-12	0.65±1.69	2.2

*- Larval stage

**- Consisting of *D. formosus, D, dulkeiti, D. baueri, D. arquatus, D. inexpectatus and Gyrodactylus kobayashii*

***- Copepodid stage

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Table 2: The prevalence, mean intensity, abundance and range of parasites of *C. gibelio* in males and females.

Parasite sex	***Diplostomum**** Prevalence(%) Mean±SD Abundance±SD Range	***Trichodina*** Prevalence(%) Mean±SD Abundance±SD Range	***Ichthyophthirius*** Prevalence(%) Mean±SD Abundance±SD Range	**Monogeneans**** Prevalence(%) Mean±SD Abundance±SD Range	***Lernaea***** Prevalence(%) Mean±SD Abundance±SD Range	***Raphidascaris**** Prevalence(%) Mean±SD Abundance±SD Range
Male (N=8)	87.5 3.0±1.73 2.63±1.92 1-6	25 5.0±0 1.25±2.31 5	50 15.0±8.69 7.5±10.36 1-24	100 49.88±52.23 49.88±52.23 1-137	12.5 1.0± - 0.125±0.35 1	25 1.5±0.71 0.38±0.74 1-2
Female (N=77)	57.14 3.45±4.53 1.97±3.81 1-27	14.29 29.91±27.72 4.27±14.56 2-65	28.57 3.73±4.32 1.06±2.83 1-21	87.01 22.34±29.80 19.44±28.78 1-50	1.3 2.0± - 0.03±0.23 2	25.97 1.95±1.64 0.51±1.19 1-6

*- Larval stage

**- Consisting of *D. formosus, D, dulkeiti, D. baueri, D. arquatus, D. inexpectatus and Gyrodactylus kobayashii*

***- Copepodid stage

According to Table 2, the prevalence of parasites in females and males had varying values, but the differences between them were not significant (Z test, *p>.05*). It was also true for mean intensity of infection and abundance of these parasites (Mann Whitney U test, *p>.05*).

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Table 3: The prevalence, mean intensity, abundance and range of parasites of *C. gibelio* in different seasons.

Parasite Season	***Diplostomum**** **Prevalence(%)** **Mean±SD** **Abundance±SD** **Range**	***Trichodina*** **Prevalence (%)** **Mean±SD** **Abundance±SD** **Range**	***Ichthyophthirius*** **Prevalence (%)** **Mean±SD** **Abundance±SD** **Range**	**Monogeneans**** **Prevalence(%)** **Mean±SD** **Abundance±SD** **Range**	***Lernaea****** **Prevalence (%)** **Mean±SD** **Abundance±SD** **Range**	***Raphidascars**** **Prevalence (%)** **Mean±SD** **Abundance±SD** **Range**
Summer (N=27)	40.74 2.0±1.54 0.81±1.42 1-5	11.11 19.0±22.87 2.11±8.79 2-45	22.22 4.0±5.44 0.89±2.93 1-15	77.78 11.0±18.56 8.6±16.9 1-85	3.7 2.0± - 0.15±0.53 2	14.81 2.0±2.0 0.37±1.04 1-5
Autumn (N=48)	68.75 4.0±5.0 2.75±4.53 1-27	8.33 33.5±33.35 2.79±12.63 4-65	41.67 5.8±7.13 2.42±5.38 1-24	91.67 30.91±39.68 28.33±38.92 1-150	2.08 1.0± - 0.02±0.14 1	27.08 1.92±1.66 0.52±1.21 1-6
Winter (N=15)	60 2.56±2.42 1.53±2.13 1-7	46.67 21.86±26.65 10.2±20.77 5-65	13.33 4.5±0.71 0.6±1.59 4-5	100 26.13±16.95 26.13±16.95 1-50	0	53.33 3.25±3.77 1.73±3.15 1-12

*- Larval stage

**- Consisting of *D. formosus, D, dulkeiti, D. baueri, D. arquatus, D. inexpectatus and Gyrodactylus kobayashii*

***- Copepodid stage

According to Table 3, the mean intensity and abundance of monogeneans in autumn and winter were significantly higher than in summer (Kruskal Wallis test, X^2=9.918, df=3, $p<.05$; X^2=16.296, df= 3, $p<.05$ respectively).

The abundance of *D. spathaceum*, *Trichodina sp*., *I. multifiliis* and also *R. acus* had significantly differences between these seasons (KW test, X^2 = 10.347, 13.285, 10.376 and 7.810 respectively, df = 3, $p<.05$)

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Table 4: The prevalence, mean intensity, abundance and range of parasites of *C. auratus gibelio* in different age groups.

Fish Age	***Diplostomum**** Prevalence(%) Mean±SD Abundance±SD Range	***Trichodina*** Prevalence(%) Mean±SD Abundance±SD Range	***Ichthyophthirius*** Prevalence(%) Mean±SD Abundance±SD Range	**Monogeneans**** Prevalence(%) Mean±SD Abundance±SD Range	***Lernaea***** Prevalence(%) Mean±SD Abundance±SD Range	***Raphidascaris**** Prevalence(%) Mean±SD Abundance±SD Range
0+ (N=22)	27.27 2.17±1.94 0.59±1.37 1-6	0	22.73 3.0±2.28 0.68±1.70 1-7	81.82 13.89±13.61 8.6±16.9 1-85	0	13.64 2.0±1.73 0.27±0.88 1-4
1+ (N=21)	47.62 2.4±1.65 1.14±1.65 1-5	14.29 24.67±30.75 3.52±13.14 4-60	19.05 5.75±6.40 1.1±3.39 1-15	90.48 12.42±11.01 11.24±11.09 1-32	4.76 2.0± - 0.095±0.44 2	14.29 1.0± 0.0 0.14±0.36 1
2+ (N=18)	72.22 1.77±1.36 1.28±1.41 1-5	38.89 35.14±28.99 13.67±24.64 6-65	22.22 3.0±2.16 0.67±1.57 1-6	88.89 27.25±34.16 24.22±33.27 1-122	0	33.33 1.5±1.22 0.5±0.99 1-4
3< (N=20)	80 6.06±6.62 4.85±6.38 1-27	15 6.33±5.13 0.95±2.86 2-12	50 8.9±9.02 4.45±7.71 1-24	100 51.2±45.4 51.2±45.4 2-150	5 1.0± - 0.15±0.49 1	55 3.55±3.33 2.05±2.98 1-12

*- Larval stage

**- Consisting of *D. formosus, D, dulkeiti, D. baueri, D. arquatus, D. inexpectatus and Gyrodactylus kobayashii*

***- Copepodid stage

According to Table 4, the mean intensity of *D. spathaceum,* monogeneans and *R. acus* in older age groups were significantly higher than in smaller ones (KW test, X^2=14.250, 16.295 and 11.567 respectively, df = 5, *p<.05*). The abundance of *D. spathaceum, Trichodina* sp.*,* monogeneans and *R. acus* had significantly differences between these groups (KW test, X^2 = 18.534, 15.201, 19.278 and 18. 068 respectively, df = 5, *p<.05*).

In the present study, It was found that the mean intensity of *D. spathaceum* and monogeneans is significantly higher in larger length groups than in smaller ones (KW test, X^2 = 44.823, df = 23, *p<.05* for monogeneans; KW test, X^2 = 33.507, df = 21, *p<.05* for *D. spathaceum*). It was also true for abundance of monogeneans (KW test, X^2 = 50.169, df = 24, *p<.05*).

It was also found that the mean intensity of parasites in different weight groups had varying values, but the differences between them were not significant (KW test, *p>.05*). It was not true for abundance values of *Trichodina* sp. (KW test, X^2= 86.109, df = 66

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$p<.05$) and *L. cyprinacea* larvae (KW test, X^2= 89, df = 66 $p<.05$).

It was also found that the mean intensity of parasites in different catch points (east, central part and west) had varying values, but the differences between them were not significant (KW test, $p>.05$). It was also true for abundance values of these parasites except for monogeneans (KW test, X^2= 7.268, df = 2 $p<.05$) and *R. acus* (KW test, X^2= 6.949, df = 2 $p<.05$).

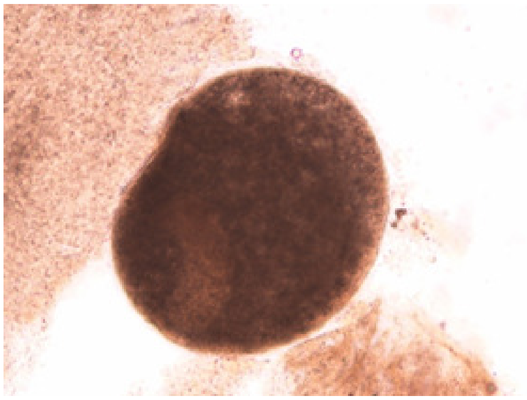

Figure 1: *Ichthyophthirius multifiliis* (50x)

Figure 2: *Trichodina* sp. (165x)

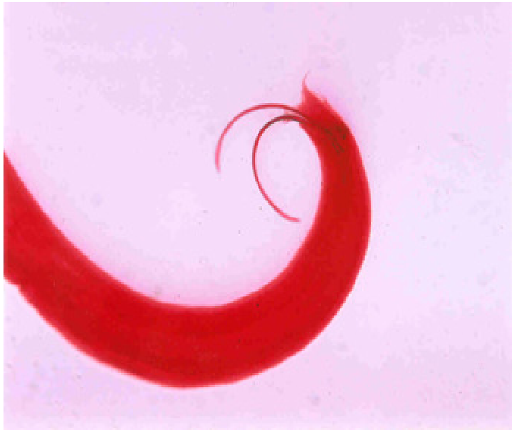

Figure 3: *Raphidascaris acus* (posterior part) (95x)

Figure 4: *Diplostomum spathaceum* larvae (40x)

Figure 5: The attachment apparatus of *Dactylogyrus formosus* (192x)

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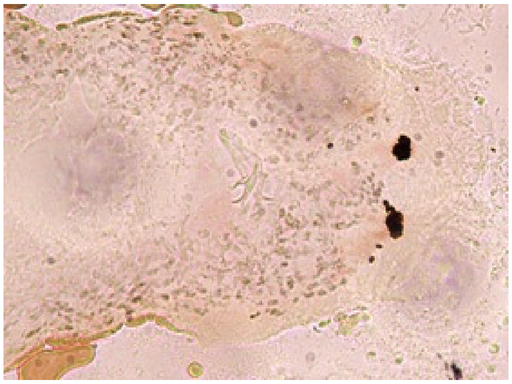

Figure 6: The copulatory organ of *Dactylogyrus formosus* (168x)

Figure 9: The attachment apparatus of *Dactylogyrus inexpectatus* (196x)

Figure 7: The attachment apparatus of *Dactylogyrus baueri* (115x)

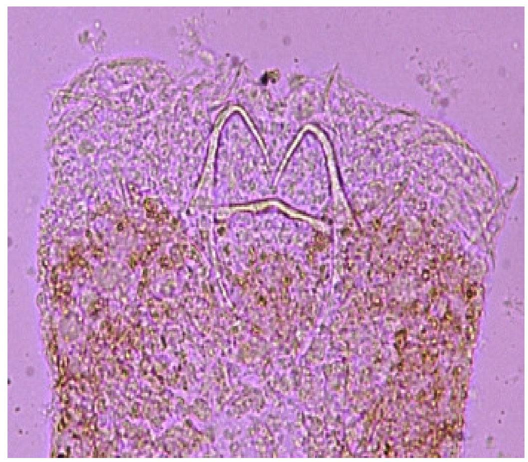

Figure 10: The copulatory organ of *Dactylogyrus inexpectatus* (190x)

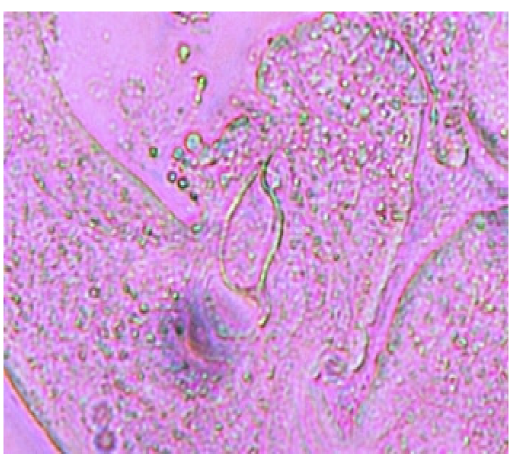

Figure 8: The copulatory organ of *Dactylogyrus baueri* (105x)

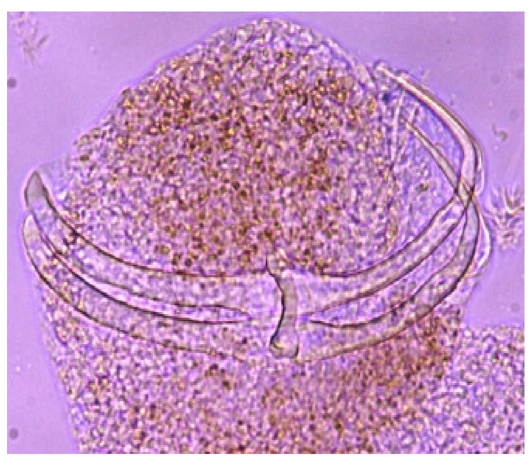

Figure 11: The attachment apparatus of *Dactylogyrus arquatus* (308x)

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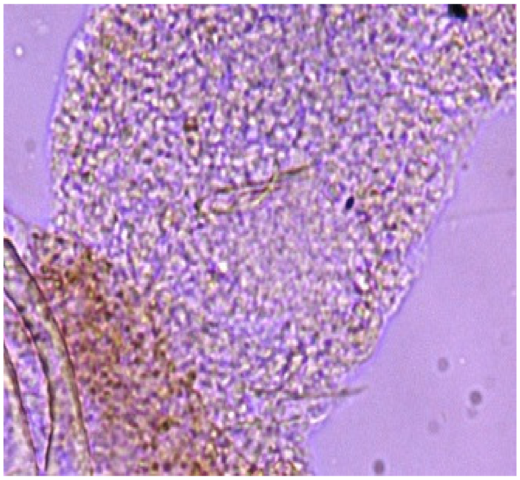

Figure 12: The copulatory organ of *Dactylogyrus arquatus* (160x)

Figure 13: *Dactylogyrus dulkeiti* (80X)

Figure 14: Central hook complex of *Gyrodactylus kobayashii* (186X)

Figure 15: Copepodid stage of *Lernaea cyprinaceae* (156X)

Discussion

Sattari (1996) reported 4 parasite species from Prussian carp consisting of *R. acus, D. spathaceum* larvae, *D. extensus* and *Gyrodactylus* sp. in Anzali wetland. In the other studies, six monogenean species including *D. formosus*, *D. baueri*, *D. extensus, D. vastator, D. wegeneri* and *Gyrodactylus prostae* were reported from Prussian carp in Iran (Jalali and Molnar, 1990; Jalali, 1995; Shamsi and Jalali, 1997). In addition, Khara et al. (2005) recovered two parasite species from Prussian carp consisting of *D. spathaceum* and *Dactylogyrus* sp. in Amirkelayeh wetland. Khara et al. (2011) also found these two parasite species from Prussian carp in Boojagh wetland.

In the present study, 11 parasite species consisting of two protozoans: *I. multifiliis* and *Trichodina* sp.; one nematodes: larvae of *R. acus*; one trematode: metacercaria of *D. spathaceum*; six monogeneans: *D. formosus, D, dulkeiti, D. baueri, D. arquatus, D.*

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inexpectatus and Gyrodactylus kobayashii; and one crustacean: copepodid stage of *Lernaea cyprinacea* were recovered from the Prussian carp. The occurrence of *D. inexpectatus* in *C. gibelio* is reported for the first time in Iran.

In the scientific literatures, there are reports that numerous species of piscivorous fishes belonging to various families might be the hosts of adult *R.acus*, but the principal definitive host of *R.acus* is pike (*Esox lucius*) (type host) and less frequently the brown trout (*Salmo trutta* m. *fario*). The larvae of *R. acus* occur in a number of fish species of various families, serving as intermediate or paratenic hosts (Moravec, 1994). Based on Sattari, (1996) *R. acus* has been previously reported from *E. lucius* in Anzali wetland, and occurrence of its larvae has also been reported from *Tinca tinca*, *C. gibelio* and *Abramis brama orientalis*. According to Sattari, (1996) the prevalence of *R. acus* in *E. lucius* was high (84%), while its larvae had low prevalence (2.4%) in *C. gibelio.* In the present study, the nematode was found in *C. gibelio* with higher prevalence (27.78%), mean intensity of infection (2.36±2.53) and abundance (0.65±1.69).

The occurrence of *D. spathaceum* metacercariae, a trematode, has been reported from several fish species in the southern part of the Caspian Sea including *T. tinca, C. gibelio*, *Cyprinus carpio*, *A. brama orientalis*, *E. lucius*, *Perca fluviatilis* and *Hypophthalmichthys molitrix*, *Vimba vimba persa, Chalcalburnus chalcoides* from Anzali wetland (Sattari, 1996; Sattari et al., 2005), *Rutilus rutilus caspius*, *Abramis bjoerkna*, *Scardinius erythrophthalmus* from Boojagh wetland by Khara et al., (2011) and *S. glanis* from Amirkelayeh wetland by Khara et al., (2005). Sattari (1996) reported *D. spathaceum* metacercariae, in the eyes of *C. gibelio* with high prevalence (95.2%), mean intensity of infection (7.7 ± 5.1) and abundance (7.2 ± 2.1). However, in the present study, this parasite was found in the eyes of the same fish with lower prevalence (58.89%), mean intensity of infection (3.34±4.17) and abundance (1.97 ± 3.59). As monogeneans are known to have relatively strict host specificity, the large number of endemic fishes suggests the existence of several new monogenean species. To date, 92 monogeneans were reported from freshwater and saltwater of Iran. In the present study, *D. inexpectatus* is reporting for the first time from fishes of Iran.

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