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Molecular identification of *Argulus japonicus* in red cap oranda goldfish (*Carassius auratus*) in Multan, Pakistan

Warda KHAN[1], Muhammad SAFDAR[2], Yasmeen JUNEJO[3]

[1]Department of Biology, Virtual University of Pakistan, Lahore 54000, Pakistan
[2]Department of Breeding and Genetics, Cholistan University of Veterinary and Animal Sciences, Bahawalpur 63100, Pakistan
[3]Department of Physiology and Biochemistry, Cholistan University of Veterinary and Animal 19 Sciences, Bahawalpur 63100, Pakistan
safdar@cuvas.edu.pk

Abstract

Fish aquariums provide the foremost characteristics of morphology and identification of the genus Argulus (Crustacea: Branchiura). Member of genus Argulus, or fish louse are ectoparasites of fresh-water fishes. The present study was investigated the molecular analysis of *Argulus japonicus*. The work was done by collecting samples from three different regions of Multan, Pakistan. DNA was extracted following protocol specified for for the isolation of DNA. The quality of DNA was checked by agarose gel electrophoresis in 0. 8% agarose gel for 45 minutes. The PCR was optimized by amplifying the DNA with species-specific primers. Further sensitivity of PCR was done by evaluating the identified DNA of *Argulus japonicus* by diluting the DNA up to 0. 01 ng. The rate of prevalence of *Argulus japonicus* at Bosan Road was 10%, Chungi no. 09 was 62% and at Dera Adda 76%. This study revealed that *Argulus japonicus* is prevelented in the gold fish in Multan region. This study may help with the management of *Argulus japonicus* parasite in fish aquarium at Multan, Pakistan.

Keywords: *Argulus japonicus*, Redcap oranda goldfish, Molecular identification.

1. Introduction

Discovery of the fish louse, *Argulus japonicus* Thiele (Crustacea: Branchiura), in Britainby (Rushton-Mellor, 1992) in the journal, “Aquaculture and Fisheries Management”. The Argulidae is an remarkably attractive and graceful little creature; and as it can depart the fish through which it nourished, and swim without stinting in the water, there are many opportunities for watching its gambols through its native element" (Baird, 1850). The argulids mostly parasitize the fresh water fish but few have been reported from marine fish (Jafri & Mahar, 2009; Yıldız & Kumantas, 2002) stated that a trench in Europe was found with thousands Argulus sp. Fish louse, are 5-10 mm in size and found nearly worldwide with about 150 species known at present. (Tokşen, 2006). *Argulus inducus, A. japonicus, and A. siamenses* have been reported to cause mortality in major and Chinese carps (Iqbal, Mumtaz, & Sajjad, 2013; Feroz Khan, Sanker, & Prasanna Kumar, 2016; Williams, 1997) recently examined the effectiveness of a chitin inhibiting treatment (diflubenzuron) for the treatment of *Argulus* infestations and particularly the success from oral administration of these chemicals.

Since animal welfare concerned by legislation firstly introduced, the first act (2006) by Animal welfare includes fishes (Council 2009) The Act includes the “5 freedoms” of animal welfare related to fishes: Discomfort, injury, normal behave, thirst and hunger, stress. Due to these

factors parasites attacks fishes in aquaculture freshwater habitat. Injury or disease can be caused by some parasites (Rushton & Mellor 1992) first recorded *Argulus japonicus* common name Japanese fish louse (Maxillopod, Crustacea). *Argulus japonicus* now may be widely spread in all over the world (Brewster 2016).

This study aimed to identify the *Argulus japonicus* in gold ornamental fish in aquarium of Multan regions, Pakistan by PCR.

2. Material and Methods

Sample collection

This study was carried out between April 2018 till September 2018. Sample of Argulus specie was collected from almost 15 different aquarium houses situated in Multan region. The sample of Argulus species separated from red cap oranda gold fish (*Crassius auratus*) and taken in falcon tube. The sample was taken to laboratory and 70% of absolute ethanol was used to preserve samples of genus Argulus for further study.

DNA Isolation

The preserved sample of specie of Argulidae was made ethanol free by repeated washing with saline. Method for isolation of DNA from genus Argulus is Add 250 µl of solution 1(TE buffer, 50mMTris Hcl, 10mM EDTA, 6M Nacl) into 1. 5ml eppendorf tubes. Add the sample of preserved genus Argulus and homogenize the sample into the solution very well by sterile micro-pestle (crush)**.** Resuspend in protenase K (10 µl) & SDS 2% (20µl). The content of the tube was vortex and mixed gently. The content of tube was gently inverted and Incubated at 50º C for 2 hours in water bath. Resuspended in250 µl (25:24:1) Phenol: chloroform: IAA proportional solution were mix well by using pipette. The tube was then centrifuged at 13000rpm for 10 min**.** aqueous phase were collected into new tube**.** Above steps were performed twice for centrifugation, by adding proportion of phenol: chloroform: IAA. Then content of sample was suspended in 700µl Chloroform and mix well by pipetting. The tube was centrifuged at 13000 rpm for 10min. Again aqueous phase was collected into new tube**.** Above step of chloroform suspension were repeated once**.** Double volume of 100% cold ethanol was added in the content of sample. After mixing, 100% cold ethanol DNA was started to precipitate. The temperature at 4ºC was maintained by running centrifuge machine empty at 3000rpm for 45 min. The tube was centrifuged at 13000rpm for 30min at 4ºC**.** Supernatant were discarded and the solution was washed by 70% ethanol**.** The tube was centrifuged at 13000rpm for 10min at room temperature (25º-27ºC). Supernatant were removed and ethanol was collected. The tube was air dried at hot plate. DNA pellet was resuspended in appropriate volume of ddH_2O (2µl). The sample was mixed and store the tube in refrigerator.

Primer design, PCR and Gel electrophoresis

The pair of primer used for *Argulus japonicus* was LCO1490: 50-GGTCAACAAATCATAAAGATATTGG-30 and HCO2198: 50-TAAACTTCAGGGTGACCAAAAAATCA-30 (Feroz Khan et al. , 2016). The conditions for amplification of *Argulus japonicus* includes introduce 94ºC heat for 1 min, at 94ºC allow 5 cycles for 30 sec, 49ºC annealing temperature for 40 sec, 1 min at 72ºC, and 10 min at 72ºC extension. The DNA samples were run at 160 volts, 400 mV for 45 minutes.

3. Results

The specificity of primers was checked and there was no cross activity as shown in figure 1. Furthermore, the sensitivity of the primers was 0. 01ng/ul of the targetted *Argulus japonicus* (Figure. 2). Additionally, the prevalence level of presence of *Argulus japonicus* was detected by molecular analysis of each sample of Argulids. The rate of prevalence of Argulus japonicus at Bosan Road was 10%, Chungi no. 09 was 62% and at DeraAdda 76% (Table 1 & figure 3).

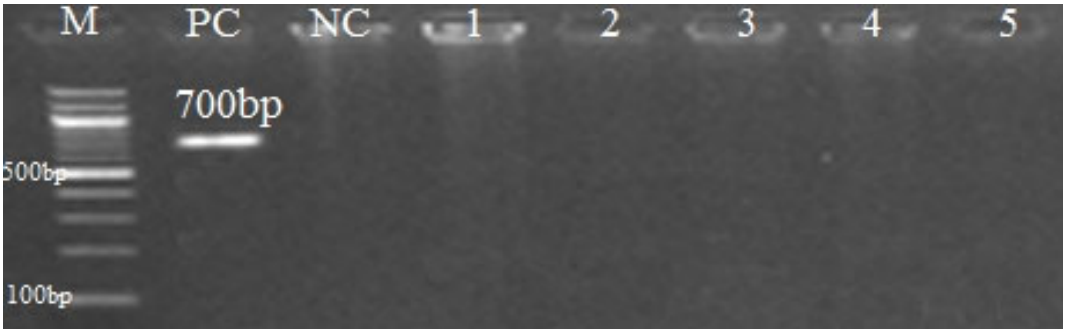

Figure. 1. Specificity of PCR assay of DNA from *Argulus japonicus* sample: M: marker, 100 bp, NC: negative control (reagents with primers without DNAs) PC; *Argulus japonicus* DNA (1) *A. foliaceus* (2) Argulus coregoni (3) Carassiusauratus (4) *H. molitrix* (5) , *C. Mrigala*

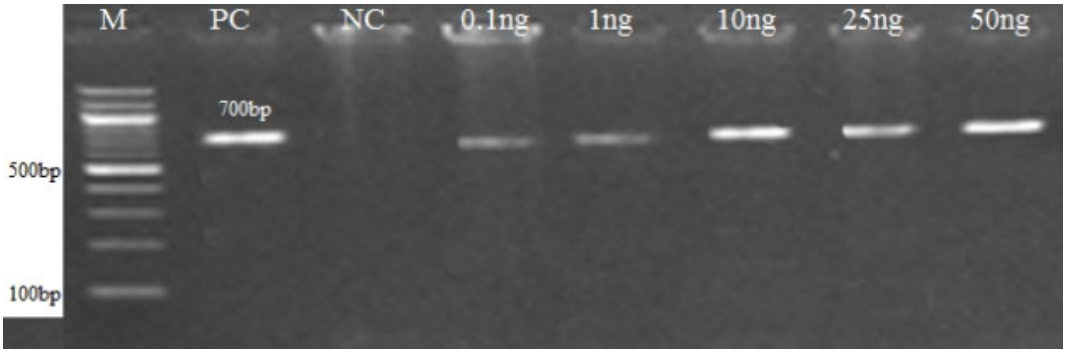

Figure. 2. Evaluation of PCR assay sensitivity for *Argulus japonicus* DNA sample; M: marker 100 bp. NC: negative control (reagents with primers without DNAs) PC; *Argulus japonicus*DNA (1) 0. 01ng (2) 0. 1ng (3) 1ng (4) 10ng (5) 25ng (6) 50ng

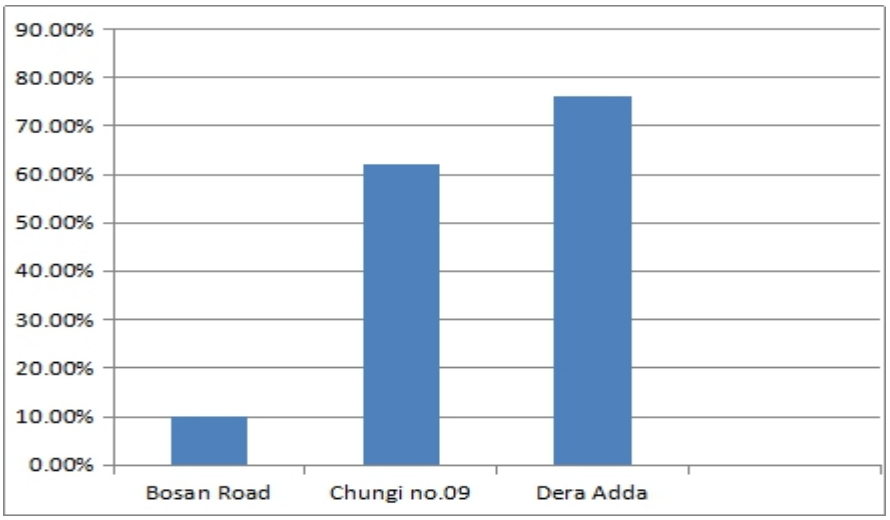

Figure 3. Prevalence by Molecular Identification of *Argulus japonicus* in Red Cap Oranda Goldfish (*Carassiusauratus*) in district Multan, Pakistan

Table. 01 Results of PCR for identification of *Argulus japonicas* collected from the different aquarium of District Multan

Sr#	*Bosan Road*	*Chungi no. 09*	*DeraAdda*
1	-	+	+
2	-	+	+
3	-	+	+
4	-	-	-
5	-	+	+
6	-	+	+
7	-	-	-
8	+	+	+
9	-	-	-
10	+	-	+

4. Discussion

Parasites of fish cause high commercial losses in both the aquaculture (Avenant-Oldewage & Everts, 2010) and fisheries industries (Nowak, 2007) and can have serious socio-economic in both developing and developed countries (Walker, Flik, & Bonga, 2004). The intimate relationship between parasites and their hosts have typically co-evolved over countless generations. Parasites are continually locked in an evolutionary arms race Red Queen Hypothesis; see (Woolhouse & Webster, 2000) with their hosts, each species constantly evolving new strategies to increase its fitness and gain an advantage over the other. We expected learning about fish parasite so we can answer this statement of problem.

The present study showed that the specificity of primers was checked and there was no cross activity as shown in figure 1. Furthermore, the sensitivity of the primers was 0. 01ng/ul of the targetted *Argulus japonicus* (Figure. 2). Additionally, the prevalence level of presence of *Argulus japonicus* was detected by molecular analysis of each sample of Argulids. The rate of prevalence of Argulus japonicus at Bosan Road was 10%, Chungi no. 09 was 62% and at DeraAdda 76% (Table 1 & Figure 3).

Host integument, immune response, stress response and secondary infections were the effects on Goldfish family by Argulus specie. Through the feeding and attachment mechanism Argulus specie causes damage to integument of Red Cap Oranda Goldfish. This can also be the result of some chemical secretion by Argulus specie or might be due to some mechanical action like scratching host integument by sharp parts of mouth. The notable immune system to Argulids infestation was localized inflammation. Inflammation was appeared as red spots on the integument of Red cap Oranda Goldfish. The blood levels increase dramatically in response to stress caused by Argulus specie.

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